The functional analysis of Th cells in DSS-induced colitis

(DSS 誘導性腸炎におけるヘルパーT 細胞の機能解析)

２０１３

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博 士 （ 医 学 ） 学 位 論 文

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1. Introduction

Inflammatory bowel disease (IBD) refers to ulcerative colitis (UC) and Crohn's disease (CD). CD and UC are the two main forms of chronic inflammatory bowel disease. CD is a transmural inflammatory disease of the mucosa with episodic progression. It can affect every part of the gastrointestinal tract from the mouth to the anus. Typical manifestations include discontinuous involvement of different segments of the gastrointestinal tract. UC is a nontransmural inflammatory disease with episodic progression that is restricted to the colon.[1] The pathogenesis of IBD remains unclear, although it is widely accepted that genetic, environmental, and immunological factors are involved.[2, 3] Importantly, T cells and their secreted cytokines are the main effectors in the induction and perpetuation of intestinal inflammation.[4] $CD4^+$ T helper (Th) cells are a subcategory of T lymphocytes that play a central role in modulating immune responses. Three major subtypes of effector T helper cells have been identified, Th1, Th2, and Th17 cells. The Th1 cells induce cellular immunity, granuloma formation, and protect against intracellular pathogen. The Th2 subset favors production of the various immunoglobulin classes that shape or help humoral immunity. Th17 cells have been

shown to participate in the development of autoimmunity, and also to play an important role in host defense against infection. These three polarized T helper subsets can be identified by the cytokines they secrete. Th1 cells produce interleukin-2 (IL-2) and interferon-g (IFN-α), Th2 cells produce IL-4, IL-5, and IL-13, while Th17 cells produce IL-17, IL-21, IL-22. Until a few years ago, naïve $CD4^+$ cells were thought to differentiate into two cell types, T helper (Th) 1 and Th2 cells. The Th1/Th2 paradigm was therefore used to differentiate the underlying immunological conditions of CD and UC. The dominant paradigm was that CD was characterized by a Th1 mucosal immune response, caused by the action of IL-12, resulting in overproduction of interferon (IFN)-α and IL-2, while UC was thought to be characterized by a Th2 response, with excess production of IL-5 and IL-13.[5, 6] More recently, a third subset of T helper cells, Th17 cells, have been described. This distinct lineage does not share developmental pathways with either Th1 or Th2 cells. Th17 cells produce IL-17, IL-22 and IL-23. IL-17 expression in the mucosa and its serum levels were increased in active IBD patients.[7, 8]

Several models of experimental colitis have been reported that demonstrate

various pathophysiological aspects of human IBD.[2] Dextran sulfate sodium (DSS)-induced colitis is a well-established animal model of mucosal inflammation for the study of IBD pathogenesis.[9, 10] DSS, one of the mucopolysaccharides, can develop the intestinal mucosal epithelial disorder. Thus, mice by drinking with DSS containing water can induce enterocolitis. DSS colitis is known as a UC model, and many studies have described UC as a Th2 disease.[11, 12] However, several studies demonstrated that DSS colitis is dependent on Th1- or Th17-mediated inflammation.[13-16] Thus, the roles of T helper cells in DSS colitis are unclear. T-bet, GATA-3, and retinoic acid-related orphan receptor gamma-t (RORγt) are known as Th1[17], Th2.[18, 19], and Th17 lineage commitment transcription factors[20, 21], respectively. We previously generated Th1 dominant (T-bet Tg) mice[22], Th2 dominant (GATA-3 Tg) mice[23], and Th17 dominant (RORγt Tg) mice.[24] In this study, we used the Th1, Th2, and Th17 dominant mice to elucidate the roles of T helper cells in DSS colitis.

2. Material and Methods

2-1. Mice

T-bet Tg, GATA-3 Tg, and RORγt Tg male mice on the C57BL/6J background and their wild-type littermates (13 weeks old) were used. In the preliminarily study, we found that 13-week-old mice could be induced DSS colitis certainly than young mice (under 8-week-old). Transgenic mice overexpressing T-bet, GATA-3, or RORγt under the control of the CD2 promoter were generated in our laboratory, as previously described.[22-24] Mice were fed a normal diet comprised of commercial laboratory chow (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and were maintained under specific pathogen-free conditions in the Laboratory Animal Resource Center of the University of Tsukuba. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals at the University of Tsukuba, and the study was approved by the Institutional Review Board of the university.

2-2. DSS-induced colitis

Experimental colitis was induced by administration of DSS (molecular weight

5,000 dalton; Wako Pure Chemicals Industries (Osaka, Japan)) for 4 or 7 days. For the DSS-treated group, mice were orally administrated 2.5% DSS in drinking water, and for the control group, mice received tap water. Mice from each group were sacrificed at day 4 or day 7.

2-3. Evaluation of DSS colitis

During the experiment, body weight, stool consistency and bleeding were monitored daily, and the modified disease activity index (DAI) was calculated (Table1). The following parameters were used for calculation: (a) weight loss (0 point = none, 1 point = 1–5% weight loss, 2 points = 5–10% weight loss, 3 points = more than 10% weight loss), (b) stool consistency (0 point = normal, 1 point = soft stools, 2 points = very soft stools, 3 points = watery stools), and (c) the date of start of blood in stool (0 point = no blood in stool, 1 point = day 7, 2 points = day 6 or day 5, 3 points = within day 4). The DAI was calculated as the total score for these parameters: the sum of weight loss, stool consistency, and day of bleeding, with the total modified DAI score ranging from 0 (unaffected) to 9 (severe colitis). On day 7, macroscopic assessment of

colitis was performed by measuring colon length. Colons were removed from the ileocecal junction to anal verge was measured.

2-4. Histopathological analysis and immunohistochemistry

Colon tissue from each mouse was fixed in 10% formalin in 0.01 M phosphate buffer (pH 7.2) and embedded in paraffin. Sections (3 μm) were stained with hematoxylin and eosin (H&E) for histopathological examination by light microscopy. We used a rabbit anti-mouse myeloperoxidase (MPO) polyclonal antibody (Thermo Scientific, Cheshire, UK) for staining of MPO-positive neutrophils, and a rat anti-mouse macrophage (F4/80) antibody (Cederlane, Burlington, ON, Canada). MPO staining and F4/80 staining were performed using Histofine Simple Stain MAX PO (rabbit) and Histofine Simple Stain MAX PO (rat) (Nichirei, Tokyo, Japan), respectively. For fluorescence staining, we used a goat anti-mouse IL-13 (R&D System, Minneapolis, MN, USA) and Alexa Fluor 546 donkey anti-goat IgG antibodies (Invitrogen Corporation, Camarillo, CA, USA). For histological analysis, the numbers of ulcers and infiltrating cell counts were measured using a BIOREVO BZ-9000 fluorescence

microscope (Keyence, Osaka, Japan).

2-5. Real time RT-PCR analysis

Total RNA was prepared from the colon of control mice or DSS-treated mice using an RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany). First-strand cDNA was synthesized using a QuantiTect Rev. Transcription Kit (Qiagen GmbH) or SuperScript III First-Strand Synthesis System (Invitrogen). *IL-4*, *IL-5*, *IL-10*, and *IL-13* mRNA levels were determined by real-time RT-PCR using a Thermal Cycler Dice Real Time System (Takara Bio Inc., Otsu, Shiga, Japan) with SYBR Green PCR Master Mix (Takara Bio Inc.). This procedure enabled the initial mRNA content of the cells to be standardized relative to the amount of *hypoxanthine phosphoribosyltransferase* (*HPRT*) mRNA.

The following specific primers were used for PCR:

IL-4-forward: 5'-GGTCTCAACCCCCAGCTAGT-3'

IL-4-reverse: 5'- GCCGATGATCTCTCTCAAGTGAT-3'

IL-5-forward: 5'- CTCTGTTGACAAGCAATGAGACG-3'

IL-5-reverse: 5'-TCTTCAGTATGTCTAGCCCCTG-3'

IL-10-forward: 5'- GCTCTTACTGACTGGCATGAG-3'

IL-10-reverse: 5'- CGCAGCTCTAGGAGCATGTG-3'

IL-13-forward: 5'-CCTGGCTCTTGCTTGCCTT-3'

IL-13-reverse: 5'-GGTCTTGTGTGATGTTGCTCA-3'

HPRT-forward: 5'-TTGTTGTTGGATATGCCCTTGACTA-3'

HPRT-reverse: 5'-AGGCAGATGGCCACAGGACTA-3'

2-6. FACS analysis

Colon cells were routinely collected 4 days after DSS administration mice. After sacrifice, mice were systemic perfusion with HBSS. After that, remove the colon, and washed with HBSS(-) quickly at 4℃. colon were cut into 5-10 cm and put on 5% FCS-HBSS(-) and shaken for 20 minutes at 136rpm on 37℃. After that, shake strongly 20 times. It was filtered through a mesh, and new 5% FCS-HBSS(-) was repeated two times. After that, cut into 5mm square the colon, and stirred 20-30 min at 37℃ to put on 5% FCS-HBSS(+) containing collagenase. It was immediately filtered through a

mesh on ice and centrifuge for 10 minutes at 1200rpm. Pellet were suspended in 5% FCS-HBSS(+) to stain surface markers. We used anti-mouse CD3-FITC, NK1.1-PE antibodies, and along with mouse IgG1-FITC as an isotype control (all BD Biosciences). The suspension was analyzed by flow cytometry. (BD LSR)

2-7. Statistical analysis

All data are expressed as means ± SEM. Multiple data comparisons were performed by using one-way analysis of variance (ANOVA). Significant differences between the groups of mice were analyzed using a Student's t-test for paired samples. *P* values <0.05 were considered statistically significant.

3. Results

3-1. GATA-3 Tg mice developed severe colitis after DSS administration

There was no significant difference in the food intake between Tg mice treated with or without DSS, and wild-type littermates treated with DSS or without DSS. Body weight was compared with the pretreatment DSS body weight (Fig. 1). There were no significant changes in control mice (Fig. 1A). In the DSS treatment groups, the body weight loss ratio in GATA-3 Tg mice was significantly more severe than that of wild-type mice from day 1 to day 7 (Fig. 1B). On day 7, the mean body weight of GATA-3 Tg mice decreased to 84.8 ± 2.3% compared with the pretreatment body weight and was significantly lower than those of the other groups (wild-type mice, 92.1 ± 1.9%; T-bet Tg mice, 92.0 ± 0.9%; RORγt Tg mice, 92.8 ± 1.4%). Next, we measured the modified DAI based on the body weight loss, stool consistency, and the blood was first present in stool. We added the day of bleeding upon the original DAI. Because, the GATA-3 Tg mice showed bleeding in the early stages. DAI was markedly higher in DSS-treated GATA-3 Tg mice compared with the other groups (Fig. 2). The mean DAI scores of the DSS-treated wild-type, T-bet Tg, GATA-3 Tg, and RORγt Tg mice were

4.8 ± 0.5, 3.3 ± 0.4, 8.1 ± 0.4, and 4.6 ± 0.4, respectively. These results indicated that GATA-3 Tg mice developed severe colitis after DSS administration compared with the other groups. In addition, another sign of disease activity noted in DSS-treated mice was colonic shortening. Intense inflammation induced abrasion of mucosal surface and a large number of ulcers and erosions. As the disorder progressed, shortening of the colon and atrophy of the intestinal mucosa were occurred. Therefore, evaluation of intestinal length is thought to be suitable to measure the degree of damage due to inflammation. However, there were not significant differences between groups. (Fig. 3)

3-2. GATA-3 Tg mice developed severe colitis in the early stages after DSS administration

Analysis of body weight changes demonstrated that the rate of weight change of GATA-3 Tg mice was more severe than for other mice from day 1 and continued to increase until day 7. On day 4, the weight change rate of GATA-3 Tg mice was significantly more severe than those of wild-type, T-bet Tg, and RORγt Tg mice (Fig. 1B). This result suggested that colitis induced by DSS in GATA-3 mice developed

earlier than in other mice. Therefore, we further studied the mice on day 4. Histological findings for the colon tissues in DSS-treated wild-type mice, GATA-3 Tg and RORγt Tg mice showed obvious manifestations of inflammatory colitis, including ulcers and infiltration of cells. Indeed, there was a prominent infiltration of cells in GATA-3 Tg mice (Fig. 4). However, T-bet mice did not develop sever ulcers. Quantification of the ulcerated area demonstrated that ulceration occurred most frequently in the colon of DSS-treated GATA-3 Tg mice (Fig. 5). These results indicated that the severe histological signs had already occurred in DSS-treated GATA-3 Tg mice on day 4.

3-3. Prominent infiltration of neutrophils and macrophages in the colon of DSS-treated GATA-3 Tg mice

Because neutrophils and macrophages cause inflammation, we measured these cell types. Immunohistochemical analysis demonstrated the infiltration of MPO-positive neutrophils in the colon of DSS-treated GATA-3 Tg mice, wild-type mice, and RORγt Tg mice (Fig. 6A and B). Furthermore, staining of macrophages revealed, infiltration in DSS-treated GATA-3 Tg mice and wild-type mice (Fig. 7A and B). From MPO- and

F4/80-positive cell count analyses, the most prominent infiltration of neutrophils and macrophages was observed in DSS-treated GATA-3 Tg mice (Fig. 6B and 7B). On the other hand, neutrophils and macrophages were not detected in DSS-treated T-bet Tg mice. These results indicated that on experimental day 4, severe pathology had occurred locally in the colon of DSS-treated GATA-3 Tg mice.

3-4. IL-13 contributed to the development of DSS-induced colitis in GATA-3 Tg mice

Because GATA-3 Tg mice developed severe DSS-induced colitis, we analyzed Th2-specific cytokines from colon tissue by measuring mRNA levels of IL-4, IL-5, IL-10, and IL-13 by real-time RT-PCR on day 4 of DSS treatment. However, we could not detect differences clearly for IL-5, and IL-10 between GATA-3 Tg mice and other groups (Fig. 8). IL-4 expression was not detectable (data not shown). RT-PCR analysis revealed that IL-13 expression in GATA-3 Tg mice was higher than for other groups and was significantly higher than in T-bet Tg and RORγt Tg mice (Fig. 9). In this analysis, higher IL-13 level was detected in control RORγt mice. However, there was

not significant difference between control RORγt and DSS-RORγt mice. Because the standard error was large, we think added number might improved this result.

Next we analyzed IL-13 expression in the colon by fluorescence staining on day 4 of DSS treatment. Many IL-13-positive cells were detected in the ulcer field of DSS-treated GATA-3 Tg mice (Fig. 10). Therefore, IL-13 might also be produced by NKT cells in GATA-3 Tg mice. We tried to study IL-13 production in NKT cells in GATA-3 Tg mice. However, we could not clearly detect overexpression of IL-13 in NKT cells from DSS-induced mice (Fig 11).

4. Discussion

Th1, Th2, and Th17 cells have been reported to play important roles in DSS coliti.[13-16, 25, 26] In particular, Th1 and Th17 cells are important in acute DSS colitis. In this study, the overexpression of GATA-3 in T cells accelerated DSS-induced acute colitis, but not the overexpression of T-bet or RORγt, which are Th1 and Th17 lineage commitment transcription factors. We have showed that T-bet Tg mice develop the downregulation of Th2.[2] Therefore, Th1 dominant T-bet Tg mice did not develop severe DSS-induced colitis.

GATA-3 is proposed to be predominantly responsible for late Th2 cellular differentiation.[18, 19] We could see the prominent infiltration of neutrophils and macrophages in the colon of DSS-treated GATA-3 Tg mice. Neutrophils and macrophages are detected as an index of acute inflammation in this experiment. DSS-induced GATA-3 Tg mice developed severe colitis and showed increased neutrophils and macrophages than other mice. Increased neutrophils and macrophages might be the results of colitis, but not induced directly by GATA-3 overexpression. Further study to define the interaction of GATA-3 with neutrophils and macrophages

may clarify the mechanisms responsible for the development of DSS-induced colitis.

Th2 cells are characterized by the production of IL-4, IL-5 and IL-13.[27] These cytokines might play important roles in the pathogenesis of DSS colitis. However, IL-13, but not IL-4 and IL-5, was detected in DSS colitis. IL-13, a Th2 cytokine, was reported to be the key effector molecule in UC.[11] In human inflammatory bowel disease, IL-13 can cause necrosis or apoptosis of epithelial cells and induce dysfunction epithelial barrier by binding with IL-13R. Kadivar et al. reported that decreased IL-13 production in the colon lamina propria cells of ulcerative colitis patients.[28] However, on the other hand, Heller et al. studied lamina propria mononuclear cells (LPMCs), isolated from surgical specimens of patients undergoing colectomy. They showed that LPMCs from patients with UC produced significantly greater amounts of IL-13 compared with controls (patients with CD). They concluded that IL-13 was an important effector cytokine in UC that impairs epithelial barrier function by affecting epithelial apoptosis, tight junctions, and restitution velocity. We could not clearly define the roles of IL-4 and IL-5, other Th2 cytokines, in DSS colitis in our study. Further studies are needed to clarify the effect of IL-4 and IL-5 on the development of DSS colitis.

Furthermore, Fuss et al. reported that UC was associated with an atypical Th2 response mediated by natural killer T (NKT) cells producing IL-13 and having cytotoxic potential for epithelial cells.[11, 29] Glycolipids from epithelial cells, bacteria, or both induce the upregulation of IL-13 receptor α2 (IL-13Rα2) on mucosal NKT cells. Autocrine IL-13 activates these cells, which expand in number and create a positive feedback loop that enhances IL-13–mediated NKT cell cytotoxicity, causing epithelial-barrier dysfunction.[30]

Currently, there is a large effort to test anti-IL-13 strategies in the clinic, involved in human IBD. Some kinds of anti-IL-13 drugs ongoing studies with compounds targeting the IL-13 pathway. To date, most of the trials in humans using anti-IL-13 agents have been done in asthma with varying degrees of success.[31] Results from trials using anti-IL-13 agents in patients with inflammatory bowel disease are not yet available.[31] The safety and efficacy of the anti-IL-13 antibody anrukinzumab is being tested in a phase 2a study in patients with mild to moderate UC. Another phase 2a study is evaluating the efficacy and safety of tralokinumab, a fully human anti-IL-13 antibody in patients with moderate to severe UC. QAX576 is also a fully human

anti-IL-13 antibody which is being studied in patients with perianal fistulas from CD. [31]

In this study, we used T-bet, GATA-3, and RORγt Tg mice. These mice were generated with the VA vector, which contained the upstream gene regulatory region and locus control region of the human CD2 gene.[32] The VA vector has been reported to direct expression of the inserted cDNA in all single-positive mature T lymphocytes of Tg mice.[32] In this study, we did not evaluate long-term DSS treatment of GATA-3 Tg mice. It is not clear how IL-13 acts in chronic DSS colitis. In the chronic phase of DSS colitis, increased expression of Th2 cytokines, IL-4 and IL-10, was previously reported.[13] Further studies are needed to clarify the effect of IL-13 on the persistence of DSS colitis and the development of chronic DSS colitis.

Many factors are important in the acute phase and chronic phase of DSS colitis.[13-16, 33] Recent studies demonstrated that DSS colitis is dependent on Th17-mediated inflammation.[13, 16] Ito et al. reported that DSS-induced IL-17 KO mice developed colitis but had better mortality rates than DSS induced in wild-type mice. These results suggest that IL-17 is important for the development of DSS colitis, but that it can be induced in the absence of IL-17. Thus, several factors interact with each

other to develop DSS colitis. In our study, Th17 dominant mice, RORγt Tg mice, did not develop a severe form of DSS colitis. Transgenic mice overexpressing RORγt under the control of the CD2 promoter induced a Th17-dominant background that might affect other cells or cytokines expression, which contributes to the development of DSS colitis. Further studies to define the interaction of IL-17 with other factors may clarify the mechanisms responsible for the development of DSS colitis.

We also expect to prevent GATA-3 KO mice from DSS-induced colitis. However, GATA-3 KO mice show various tissue developmental disorder, and they died on embryonic day 11.5. Therefore we tried to use GATA-3 cKO mice with Cre-loxP system however we had not sufficient numbers for the experiments. Our group will continue to analyze DSS colitis with GATA-3 cKO mice.

In conclusion, we observed that GATA-3 Tg mice developed more severe colitis than T-bet and RORγt Tg mice and that increased levels of IL-13 in GATA-3 Tg mice resulted in the development of DSS colitis. These results suggested that GATA-3 overexpression in T-cells and IL-13 might play important roles in the development of DSS colitis.

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7. Table

Table1. Scoring system for the modified disease activity index (DAI).

points	0	1	2	3
weight loss	none	1-5%	5-10%	>10%
stool consistency	normal	soft	very soft	watery
bleeding(day)	none	7	5~6	1~4

The DAI as the total of scores: the sum of weight loss, stool consistency, and bleeding (day), resulting in the total DAI score ranging from 0 (unaffected) to 9 (severe colitis).

8. Figure Legends

Figure 1: The time course of the body weight loss.

Body weight changes of control mice (A) and DSS treated mice (B). Body weights were measured daily. Mean body weight (% of pre body weight) are shown. c, control. d, DSS treated. W, wild-type mice. T, T-bet Tg mice. G, GATA-3 Tg mice. R, RORγt Tg mice. Significance was evaluated by Student' s t-test (*P<0.01, vs. wild-type mice; **P<0.05, vs. wild-type mice; †P<0.05, vs. T-bet Tg and RORγt Tg mice)(n, cW=7, cT=5, cG=5, cR=7, dW=7, dT=7, dG=7, dR=7).

Figure 2: Clinical assessment of DSS-induced colitis.

Modified DAI score of control and DSS treated mice. Quantification of modified DAI was calculated on the scoring system described in Material and Methods. c, control. d, DSS treated. W, wild-type mice. T, T-bet Tg mice. G, GATA-3 Tg mice. R, RORγt Tg mice. Statistical analysis was carried out using ANOVA (*P<0.01 vs. dW, dT, and dR; **P<0.01 vs. dW and P<0.05 vs. dR)(n, cW=5, cT=5, cG=5, cR=5, dW=9, dT=9, dG=9, dR=9).

Figure 3: Colon length statistic of the control and DSS-treated mice.

Colon length. After 7 days, colon lengths were measured. c, control. d, DSS treated. W, wild mice. T, T-bet Tg mice. G, GATA-3 Tg mice. R, RORγt Tg mice. Statistical analysis was carried out using ANOVA (*P<0.01)(n, cW=10, cT=6, cG=5, cR=13, dW=15, dT=9, dG=8, dR=15).

Figure 4: Histrogical analysis of acute DSS-induced colitis by H&E-stained colonic sections.

Microscopic appearance and immunohistochemical staining of intestinal tissues on day 4 of DSS treatment. Shown are the microscopic views of H&E-stained colons, (magnification ×200, scale bar 100 μm). The dot-line indicated that infiltration of cells (WT, GATA-3 Tg, RORgt Tg) and ulcer sites (WT and GATA-3 Tg).

Figure 5: The number of ulcers in the colon tissue.

Ulcer counts. On day 4 of DSS treatment, colon ulcer counts were measured. c, control. d, DSS treated. W, wild-type mice. T, T-bet Tg mice. G, GATA-3 Tg mice. R, RORγt Tg mice. Statistical analysis was carried out using ANOVA (*P<0.01, **P<0.05)(n=3, each group).

Figure 6: Histrogical analyses of acute DSS-induced colitis by MPO-stained colonic sections.

(A) Microscopic appearance and immunohistochemical staining of intestinal tissues on day 4 of DSS treatment. Shown are the microscopic views of MPO positive cells (magnification ×200, scale bar 100 μm).

(B) MPO positive cells in colon. On day 4 of DSS treatment, MPO positive cells were measured. c, control. d, DSS treated. W, wild-type mice. T, T-bet Tg mice. G, GATA-3 Tg mice. R, RORγt Tg mice. Statistical analysis was carried out using ANOVA (*P<0.01)(n=3, each group)(**P<0.01, DSS treated vs. control).

Figure 7: Histrogical analyses of acute DSS-induced colitis by F4/80-stained colonic sections.

(A) Microscopic appearance and immunohistochemical staining of intestinal tissues on day 4 of DSS treatment. Shown are the microscopic views of macrophages (magnification ×200, scale bar 100 μm).

(B) F4/80 positive cells in colon. On day 4 of DSS treatment, F4/80 positive cells were measured. c, control. d, DSS treated. W, wild-type mice. T, T-bet Tg mice. G,

GATA-3 Tg mice. R, RORγt Tg mice. Statistical analysis was carried out using ANOVA (*P<0.01)(n=3, each group)(**P<0.01, DSS treated vs. control).

Figure 8: Expression of IL-5 and IL-10 mRNA of colon.

Real time RT-PCR analysis of IL-5 and IL-10 in colon on day 4 of DSS treatment. c, control. d, DSS treated. W, wild-type mice. T, T-bet Tg mice. G, GATA-3 Tg mice. R, RORγt Tg mice.

Figure 9: Expression of IL-13 mRNA of colon.

Real time RT-PCR analysis of IL-13 in colon on day 4 of DSS treatment. c, control. d, DSS treated. W, wild-type mice. T, T-bet Tg mice. G, GATA-3 Tg mice. R, RORγt Tg mice. Statistical analysis was carried out using ANOVA (*, P<0.05. **, P<0.01) (n, cW=6, cT=6, cG=4, cR=4, dW=4, dT=4, dG=5, dR=4).

Figure 10: Histrogical analysis of acute DSS-induced colitis by IL-13-stained colonic sections.

Immunofluorescence staining of intestinal tissues on day 4 of DSS treatment. Shown

are IL-13 positive cells (A) ,and IL-13 positive cells in colon(B) (magnification ×200, scale bar 100 μm). (c, control. d, DSS treated. W, wild-type mice. G, GATA-3 Tg mice) Statistical analysis was carried out using ANOVA (*, $P < 0.01$) (n, cW=4, cG=3, dW=3, dG=4).

Figure 11: NKT cell surface expression in the colon from WT-mice and GATA-3 mice. FACS analysis of NKT cell surface expression in WT-mice and GATA-3 mice. Colon from WT-mice and GATA-3 mice stained with antibodies against CD3 and NK1.1. The percentages of NKT cells (DP) are indicated.

Figure 1

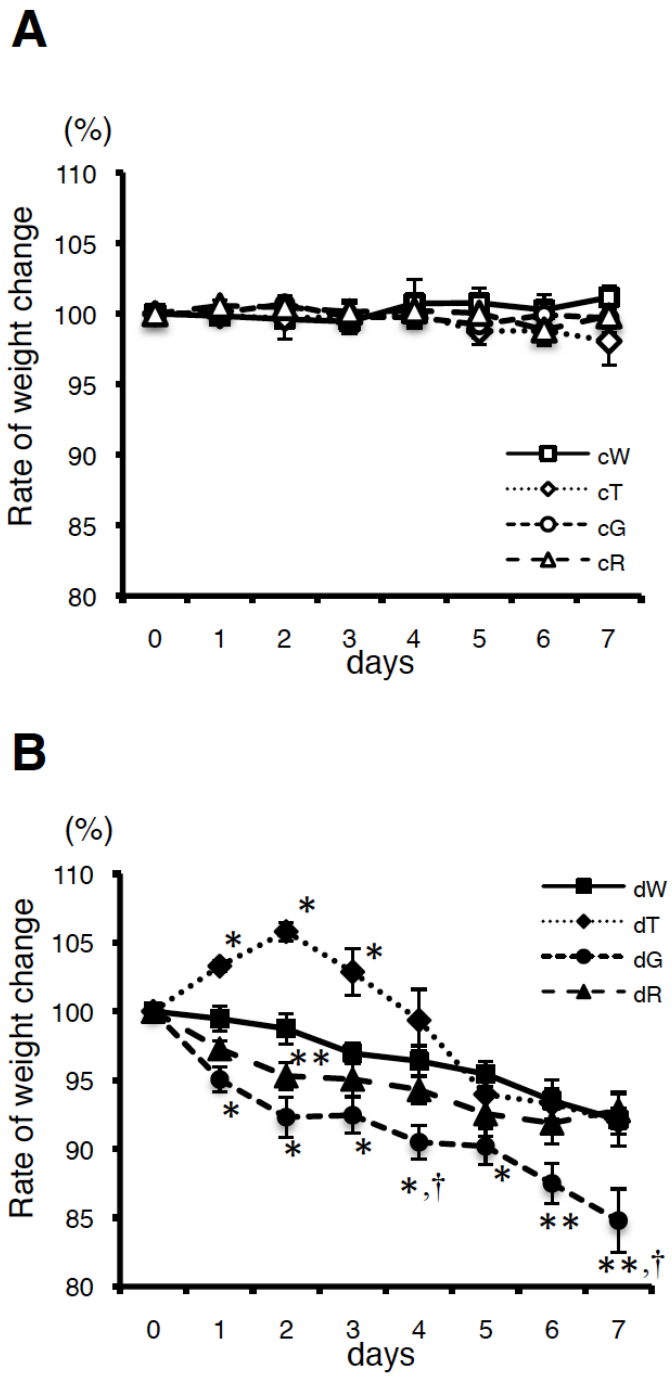
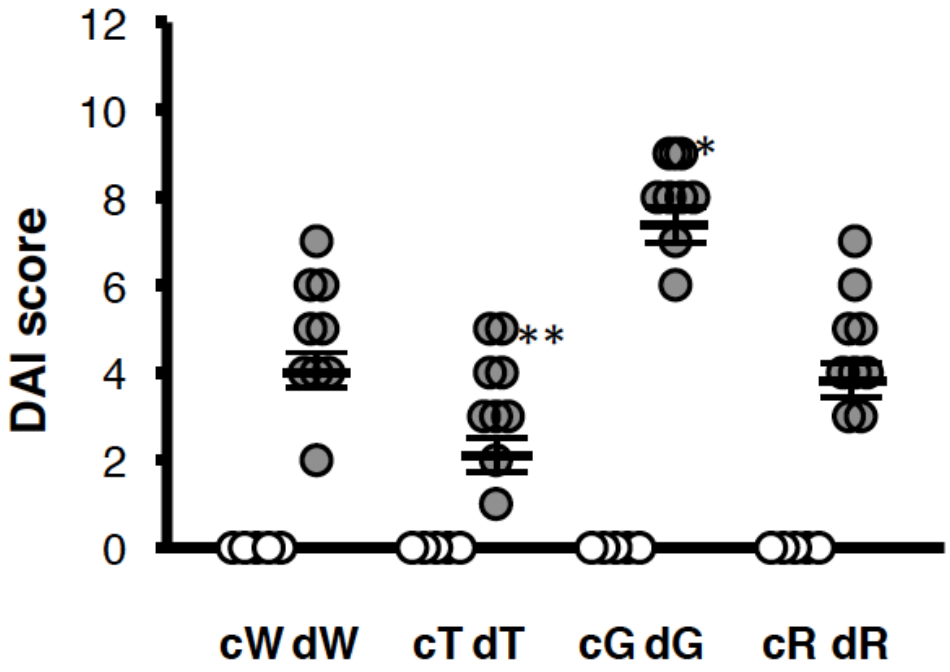

A
(%)
Rate of weight change
110
105
100
95
90
85
80
0 1 2 3 4 5 6 7
days
cW
cT
cG
cR
B
(%)
Rate of weight change
110
105
100
95
90
85
80
0 1 2 3 4 5 6 7
days
dW
dT
dG
dR
*
**
*,†
**,†

Figure 2



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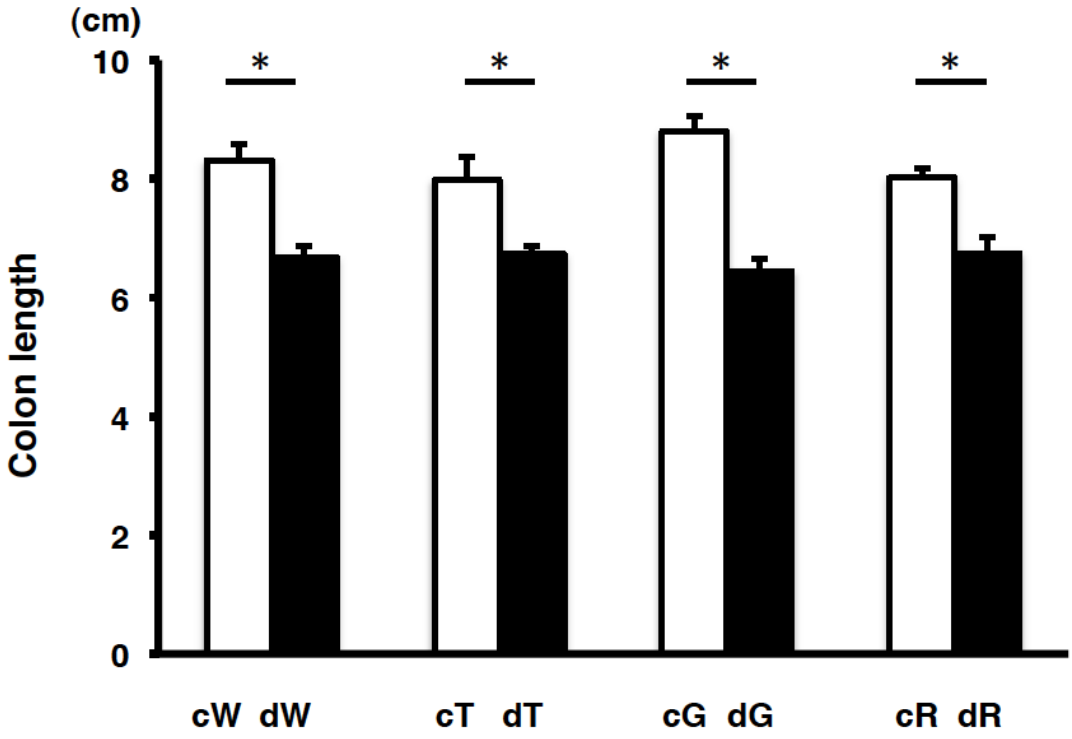

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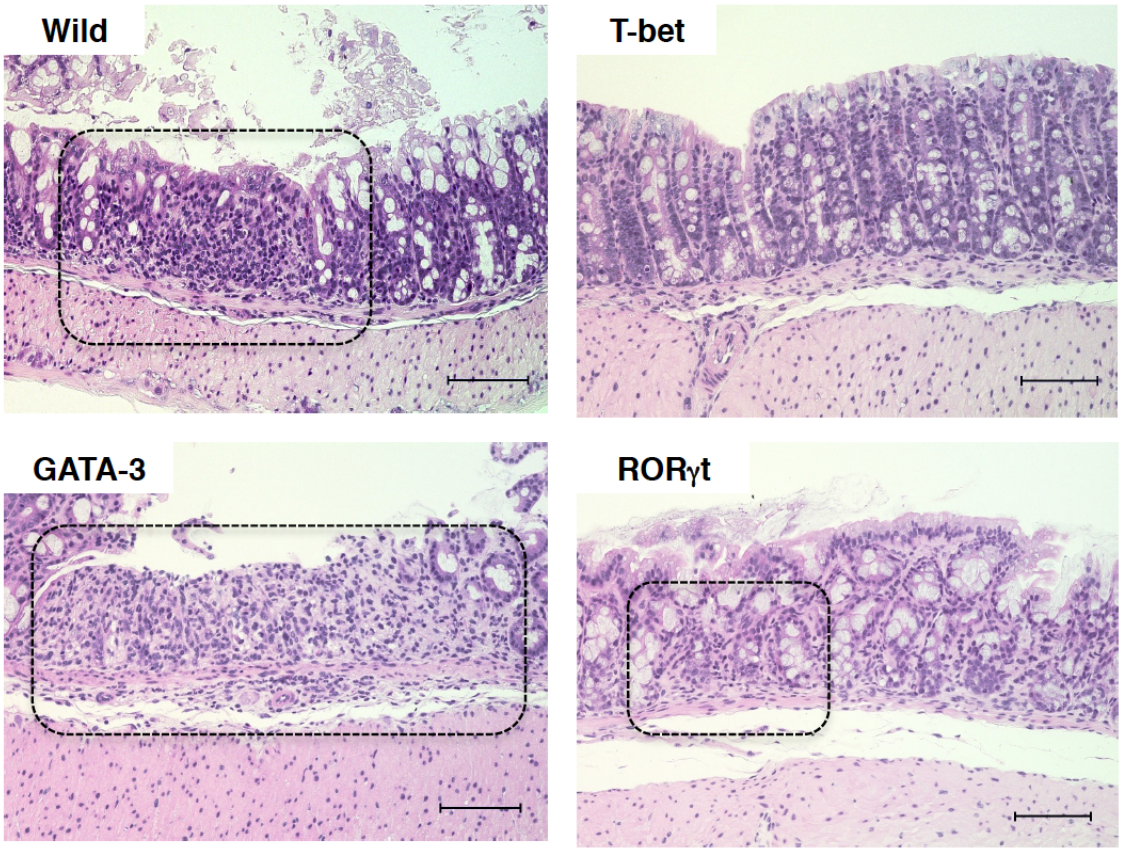

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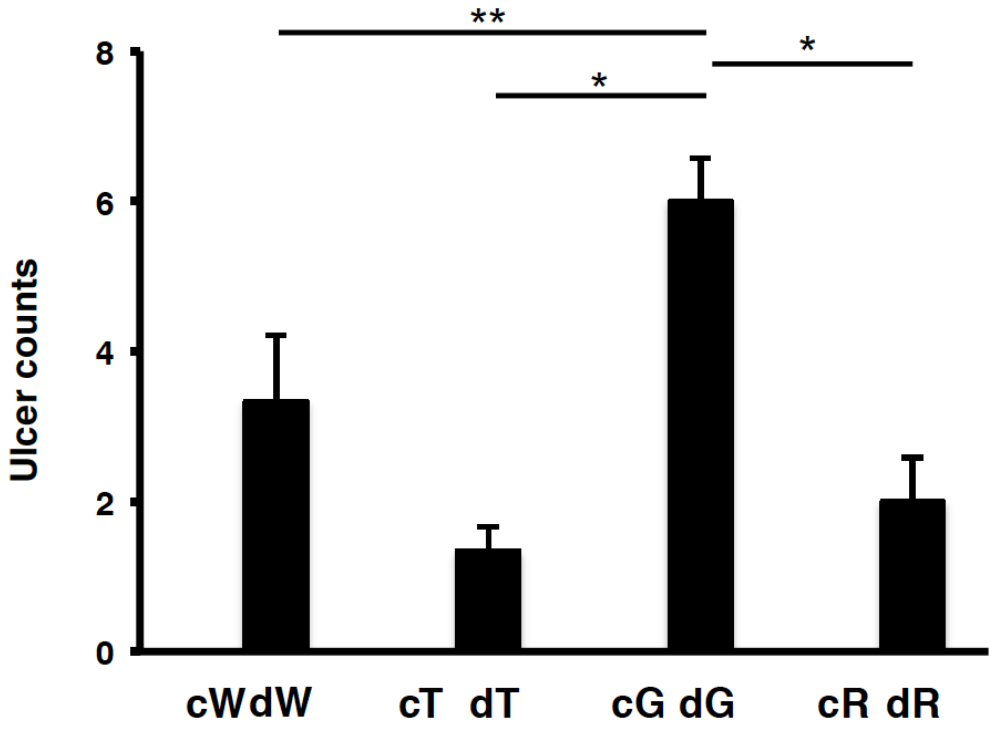
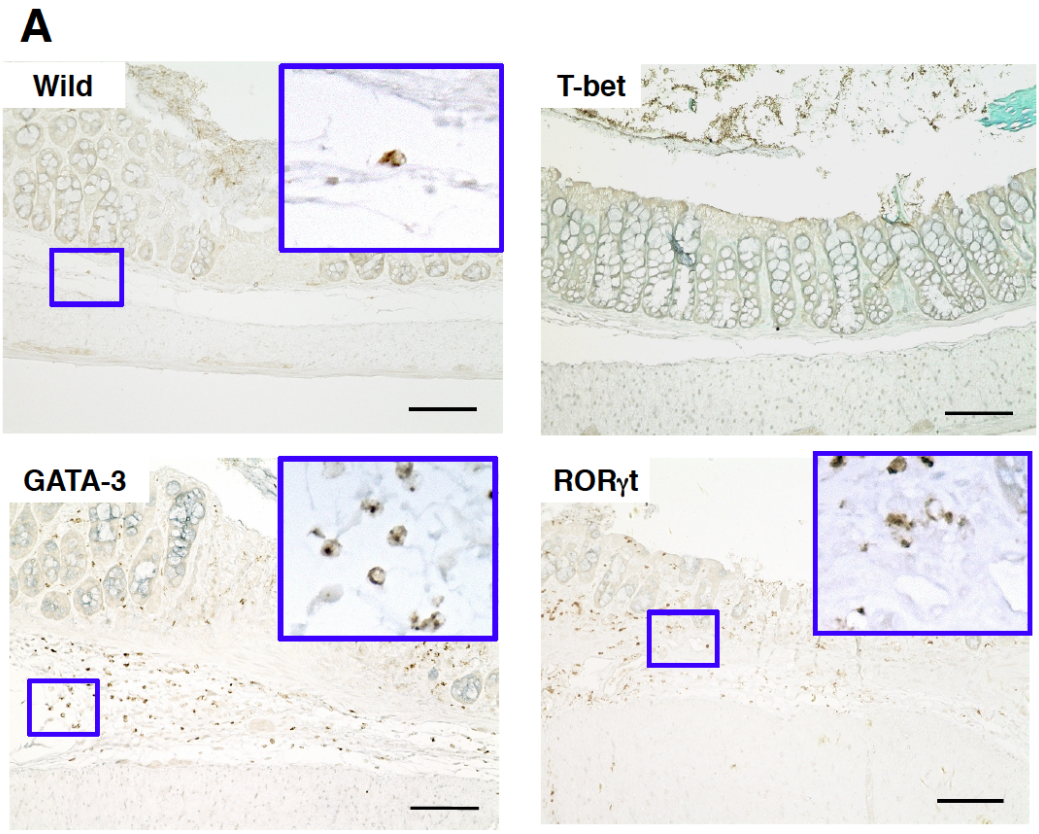


Figure 6


A
Wild
T-bet
GATA-3
RORγt

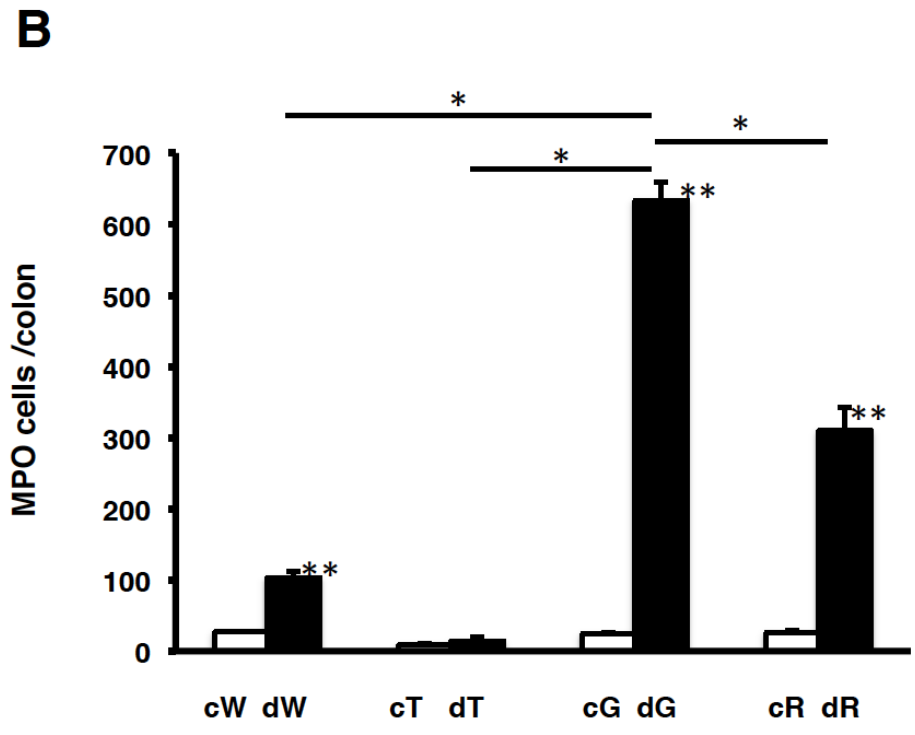
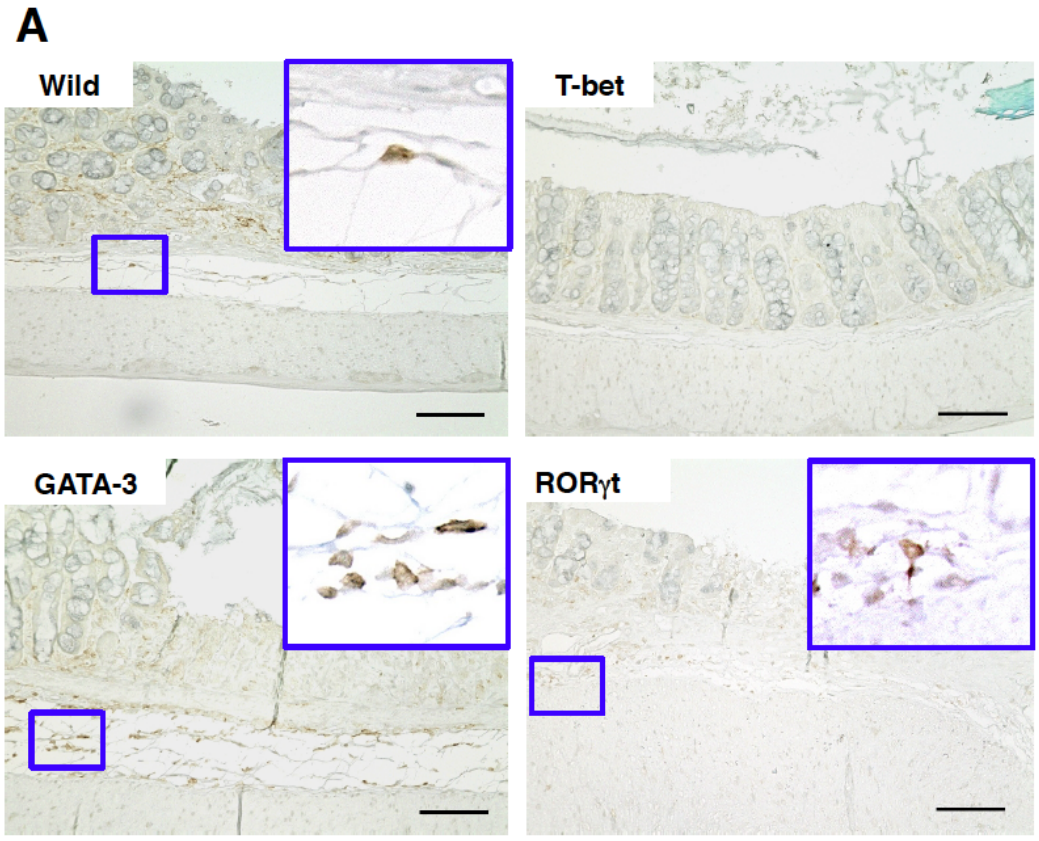

B
MPO cells /colon
700
600
500
400
300
200
100
0
cW dW
cT dT
cG dG
cR dR
*
**

Figure 7


A
Wild
T-bet
GATA-3
RORγt

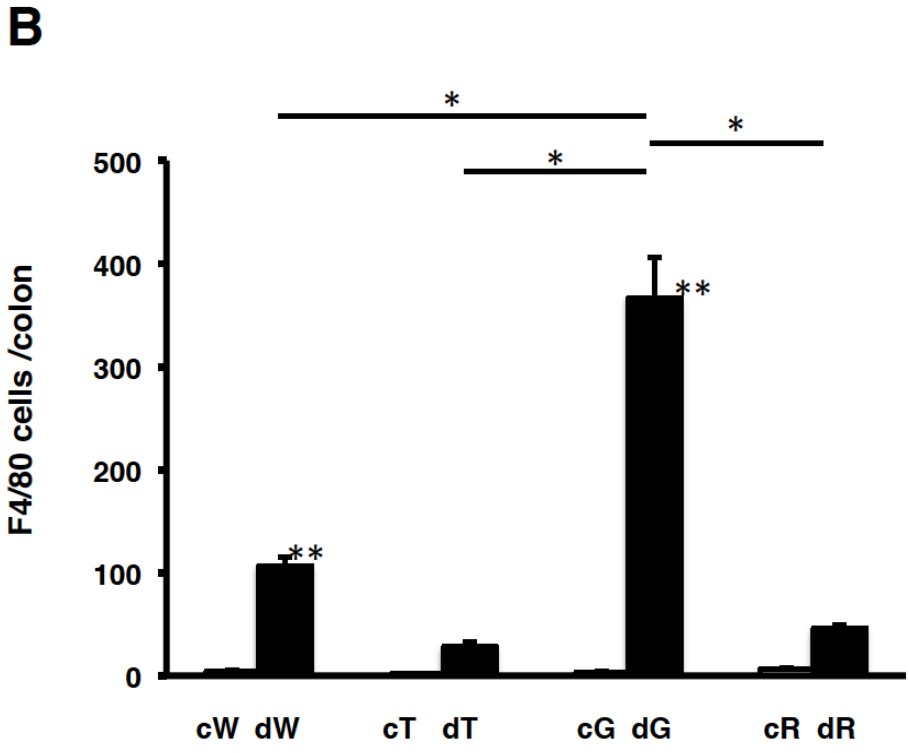

B
F4/80 cells /colon
500
400
300
200
100
0
*
*
*
**
**
cW dW
cT dT
cG dG
cR dR

Figure 8

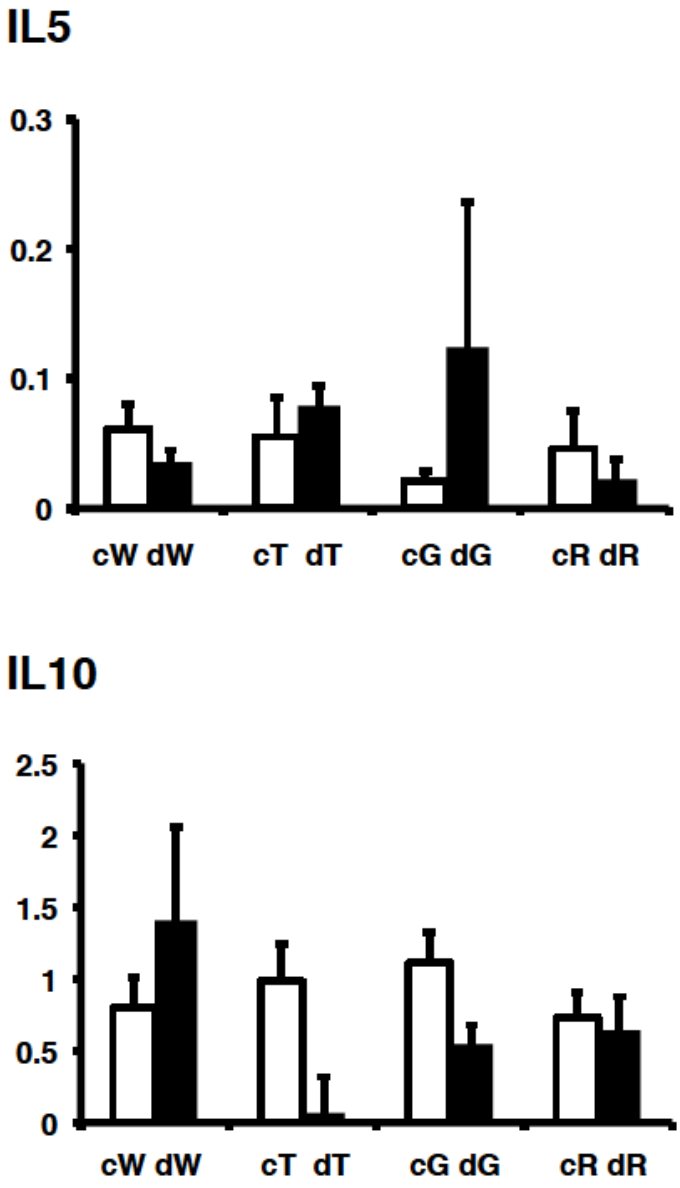

IL5
0.3
0.2
0.1
0
cW dW
cT dT
cG dG
cR dR
IL10
2.5
2
1.5
1
0.5
0
cW dW
cT dT
cG dG
cR dR

Figure 9

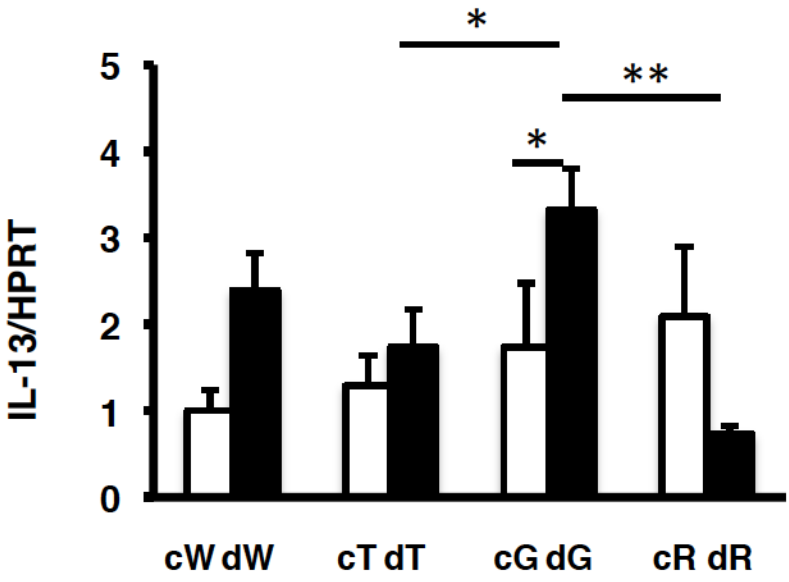


Figure 10

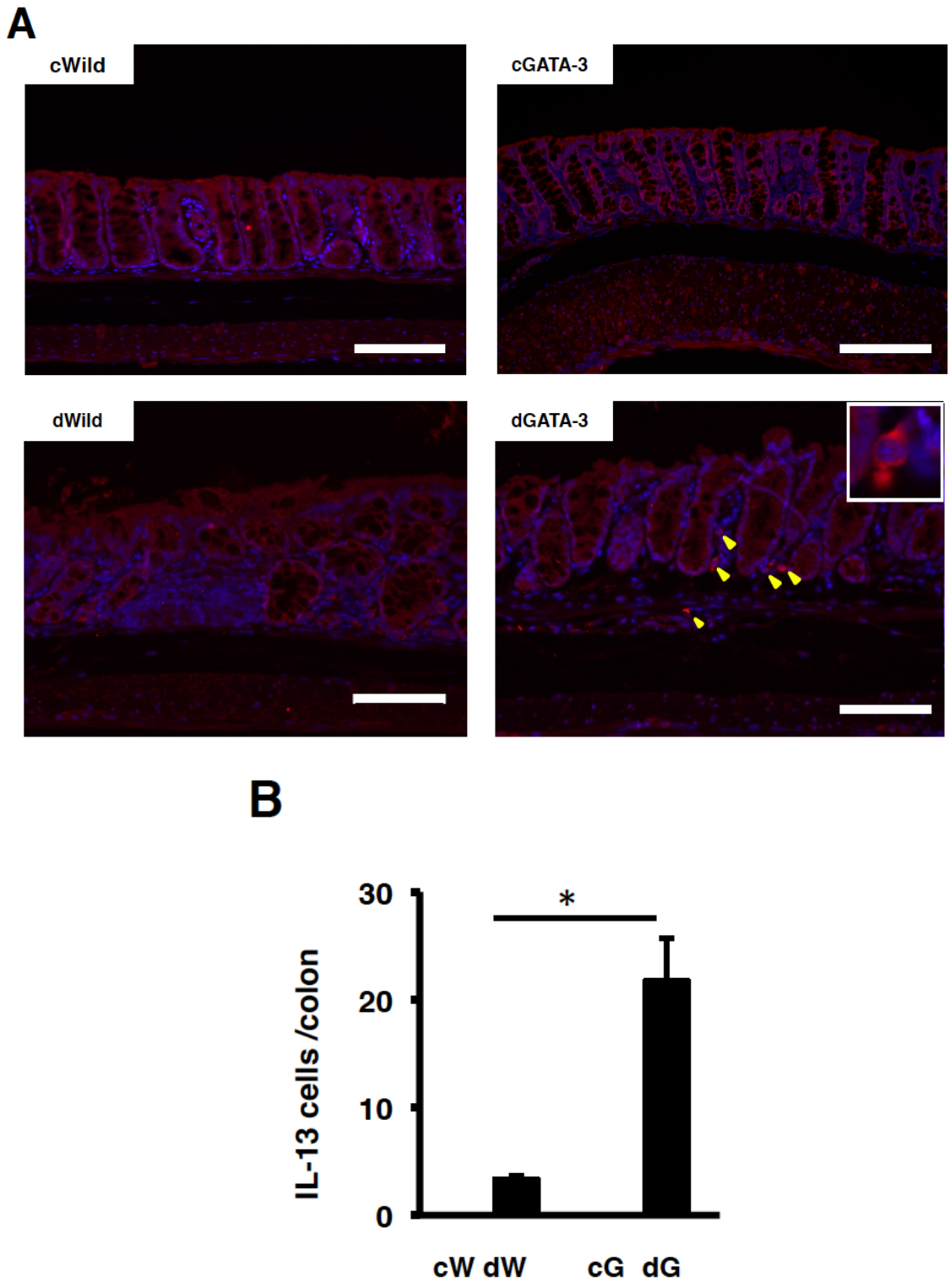
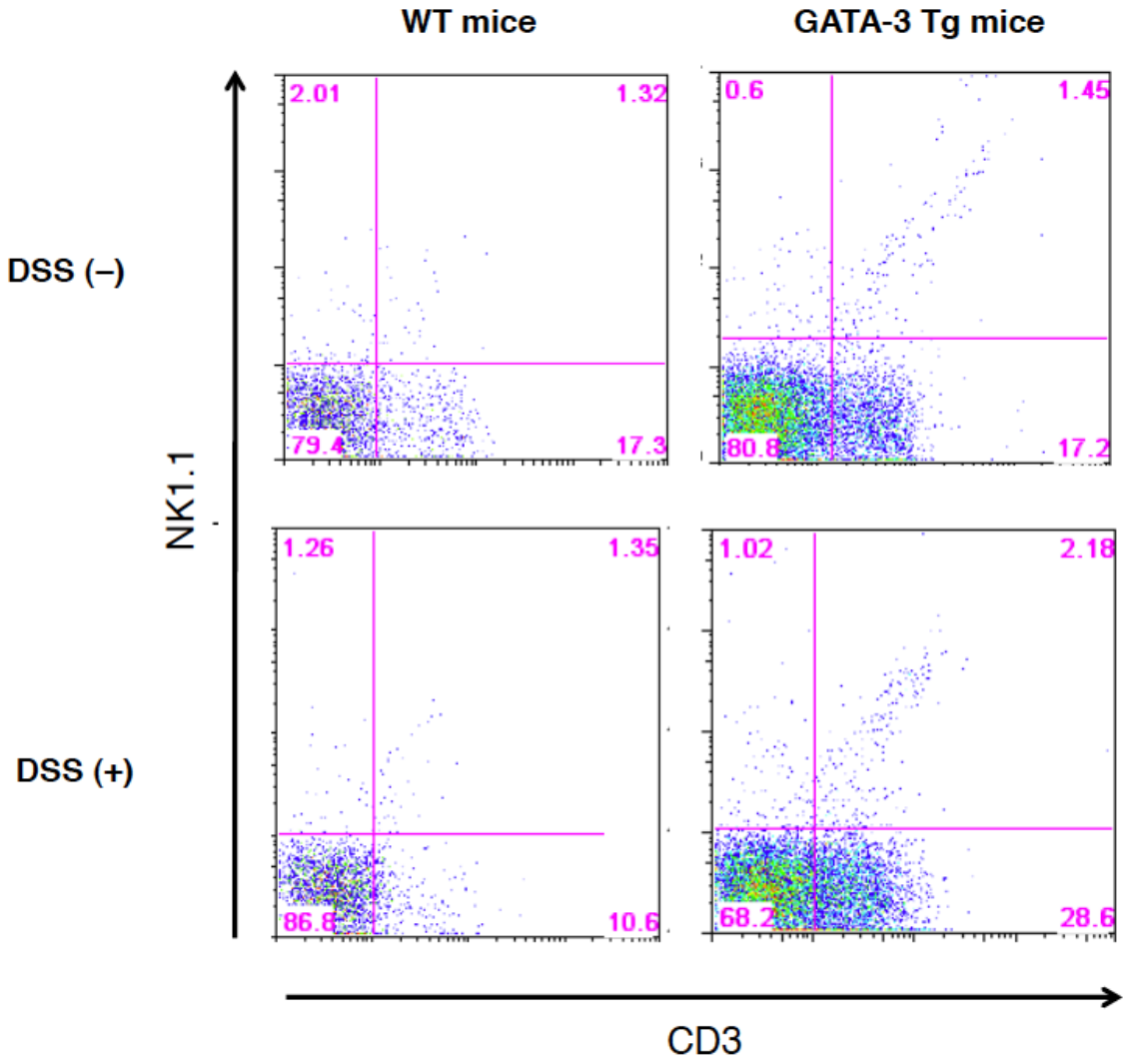

A
cWild
cGATA-3
dWild
dGATA-3
B
IL-13 cells /colon
30
20
10
0
*
cW dW
cG dG

Figure 11


WT mice
GATA-3 Tg mice
DSS (–)
DSS (+)
NK1.1
CD3
2.01
1.32
79.4
17.3
0.6
1.45
80.8
17.2
1.26
1.35
86.8
10.6
1.02
2.18
68.2
28.6