Role of eosinophils in a murine model of inflammatory bowel disease

Wang, Zi ; Adachi, Soichiro ; Kong, Lingling ; Watanabe, Daisuke ; Nakanishi, Yusuke ; Ohteki, Toshiaki ; Hoshi, Namiko ; Kodama, Yuzo

(Citation)
Biochemical and Biophysical Research Communications,511(1):99-104

(Issue Date)
2019-03-26

(Resource Type)
journal article

(Version)
Accepted Manuscript

(URL)
https://hdl.handle.net/20.500.14094/90007274

Role of Eosinophils in a Murine Model of Inflammatory Bowel Disease

Zi Wang[a], Soichiro Adachi[a], Lingling Kong[a], Daisuke Watanabe[a], Yusuke Nakanishi[b], Toshiaki Ohteki[b], Namiko Hoshi[*a], and Yuzo Kodama[a]

[a]Division of Gastroenterology, Department of Internal Medicine, Kobe University Graduate School of Medicine, Hyogo, Japan; [b]Department of Biodefense Research, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.

*Corresponding author: Division of Gastroenterology, Department of Internal Medicine, Kobe University Graduate School of Medicine, 7-5-2 Kusunoki-cho,Chu-o-ku, Kobe, Hyogo 650-0017, Japan

Phone: +81-78-382-5774, Fax: +81-78-382-6309

E-mail address: nhoshi@med.kobe-u.ac.jp

ABSTRACT

Ulcerative colitis (UC) is a form of inflammatory bowel disease (IBD), which is triggered spontaneously by unknown mechanisms and manifests as chronic and relapsing inflammatory conditions in the colon. Eosinophil infiltration is often observed in the colonic tissue of ulcerative colitis patients. However, the role of eosinophils in the disease has not been well defined. The aim of this study is to investigate the role of eosinophils in colonic inflammation using the murine model of spontaneous colitis. C-C chemokine receptor type 3 (CCR3) and interleukin (IL)-10 double knockout mice (CCR3-/-;IL-10-/-) were utilized to evaluate the function of eosinophils in colitis. The levels of colitis were evaluated by colonoscopy, histology, and real-time PCR measurements to determine expression levels of inflammatory cytokines in the colonic tissue. The levels of cytokines produced by T cells in mesenteric lymph nodes were evaluated by ELISA. There was no significant difference in endoscopic and histological scores between the groups of CCR3-/-;IL-10-/- mice and control CCR3+/-;IL-10-/- mice. There was also no significant difference in the expression levels of pro-inflammatory cytokines in the intestinal tissue between the two groups. Similar results were found for IL-17A and interferon gamma (IFN-γ) production from mesenteric lymph node-derived T cells.

Conclusion: Our data indicate that eosinophils do not play a significant role in the immunopathology of colitis in IL-10-/- mice.

Keywords: Eosinophils, CCR3, colitis, inflammatory bowel disease, ulcerative colitis, IL-10 knockout mice.

Abbreviations: UC, Ulcerative colitis; IBD, inflammatory bowel disease; CD, Crohn's disease

1. Introduction

IBD comprises two diseases—UC and Crohn's disease (CD)—that present very distinct pathological manifestations. UC affects only the colon, but CD can affect the entire alimentary tract from the mouth to the anus. The etiology of both UC and CD are yet to be elucidated, but it is thought that they are multifactorial diseases. Factors such as genetic background and deregulated immune response toward intestinal microbiota are suspected to trigger the onset of IBD [1-3]. The incidence of IBD is rising especially in Asian countries [4] making it important to investigate the mechanism of the disease.

Eosinophils can be found in the colonic lamina propria at steady state levels [5, 6]. They are not only involved in allergic intestinal disorders such as classic immunoglobulin E (IgE)-mediated food allergy [7] and eosinophilic gastroenteritis [8], but also in IBD [9]. Some studies have shown that an increased level of eosinophil granule protein was observed in the tissues and stools of IBD patients [10-12]. UC patients tend to show increased levels of T helper (Th) 2-type cytokines such as IL-4, IL-5, and IL-13 [13-15] which can be produced not only by T cells but also by other cell types including eosinophils [16]. The colonic tissue of UC patients displays infiltration of eosinophils in addition to advanced invasion of neutrophils, plasma cells, and lymphocytes [17]. These data suggest that eosinophils may play an important role in IBD; however, the role of eosinophils in IBD has not been well studied.

In this study, we aim to investigate the role of eosinophils in the chronic inflammation in the colon. CCR3 is a chemokine receptor expressed mainly on eosinophils and is known to be critically involved in the basal trafficking of eosinophils into the intestine [18]. IL-10 is a major regulatory cytokine; the IL-10 deficient mouse strain (IL-10-/-) is a widely used animal model for the study of IBD and, more specifically, UC because it spontaneously develops colitis in the presence of gut flora [19]. We made CCR3 deficient mice (CCR3-/-) in an IL-

10-/- background and evaluated whether the absence of eosinophils modifies the course of colitis.

2. Materials and methods

2.1. Experimental Mice

CCR3-/- mice (C.129S4-Ccr3<tm1Cge>/J) were purchased from the Jackson Laboratory, and crossed with C57BL/6 mice over 10 times. IL-10-/- mice purchased from the Jackson Laboratory were bred to generate CCR3+/-;IL-10-/- and CCR3-/-;IL-10-/- mice. C57BL/6 mice were purchased from CLEA (Osaka, Japan) and used as non-colitic control mice. They were kept under controlled, pathogen-free conditions. Fifteen-week-old mice were used for all experiments.

2.2. Isolation of colonic lamina propria cells and flow cytometry

Colons were excised and cleaned by removing the mesentery. Tissue was opened longitudinally, and intestinal contents were washed off. The tissue was then transferred into 50 ml conical tubes with phosphate buffered saline (PBS) supplemented with 5% fetal calf serum (FCS) and 5 mM EDTA and shaken for 20–30 min at 37 °C to remove epithelial cells. To further remove epithelium, tissue was washed several times in a petri dish with the aid of forceps. The tissue was then minced with scissors and transferred into a new 50 ml conical tube with PBS containing type VIII collagenase (Sigma-Aldrich) and DNase I (Roche). To digest the tissue, the tube was shaken for 40 min at 37 °C. Cells were transferred into a new conical tube through a 70-μm strainer and washed in PBS twice. Cells were stained with a combination of anti-CD11b (clone M1/70), anti-CD11c (clone HL3), anti-F4/80 (clone BM8), and anti-Siglec-F (clone E50-2440) after Fc receptors had been blocked. Flow cytometry was performed on BD FACSVerse (BD Bioscience) [20].

2.3. Colonoscopic Assessment

Fifteen-week-old-mice were anesthetized with isoflurane, and colonoscopy was performed. The colonoscopic findings were scored based on mucosal granularity (0, none; 1, mild; 2, moderate; and 3 severe), visibility of vascular pattern (0, normal; 1, mildly decreased; 2, moderately decreased; and 3, severely decreased), erosion or ulceration (0, normal; 1, erythema; 2, erosion; and 3, ulcer), and stool consistency (0, normal; 1, soft; 2, muddy; and 3, watery) [21, 22].

2.4. Histopathology

Colons were divided into three segments: proximal, middle, and distal. The middle colon was fixed with formalin, embedded with paraffin, and sectioned and stained with hematoxylin and eosin (H and E staining). Sections were scored on a scale of 0–3 (0, none; 1, mild; 2, moderate; 3, severe) for epithelial hyperplasia, enterocyte loss, crypt inflammation, mononuclear infiltrate, and polymorphonuclear infiltrate. The histopathology score is the summation of scores from all these criteria [22].

2.5. Isolation of RNA and Quantitative Reverse Transcription PCR

For quantitative RT-PCR, tissue was homogenized with metallic beads TRIzol® (Thermo Fisher Scientific), and total mRNA was isolated according to the manufacturer's instructions. Reverse transcription of mRNA into cDNA was performed using a cDNA synthesis kit (Applied Biosystems) and SYBR Green reagents (Applied Biosystems or Qiagen) on an ABI 7500 real-time PCR system (Applied Biosystems). The abundance of each cytokine mRNA was normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT) expression.

2.6. Mesenteric Lymph Node Cell Stimulation

After mice were sacrificed, mesenteric lymph nodes were collected and ground with sterile frosted glass slides. Cells were filtered through a 70-μm strainer to make single-cell

suspensions. They were cultured and stimulated with the plate-bound anti-CD3ε antibody for 48 hours. Culture supernatants were collected, and cytokine levels were evaluated by ELISA.

2.7. Statistical Analysis

Statistical analysis was performed with the unpaired Student's *t*-test using GraphPad Prism 6 (GraphPad Software). Data are presented as the mean ± standard error of the mean (SEM).

3. Results

3.1. Impaired Recruitment of Eosinophils into Colonic Lamina Propria of CCR3-/-;IL-10-/- Mice

CCR3 is a receptor, which recognizes several chemokines, including eotaxin, C-C motif chemokine ligand 3 (CCL3), and CCL5. CCR3 is abundantly expressed on eosinophils and shown to be involved in trafficking of eosinophils into the intestines [18]. To investigate the role of eosinophils in chronic colitis, CCR3-/- mice were crossed with IL-10-/- mice to generate CCR3-/-;IL-10-/- mice. We isolated immune cells from the colonic lamina propria and confirmed that eosinophils (CD11b+ CD11c- Siglec-F+) were almost absent in CCR3-/-;IL-10-/- mice compared to the levels seen in CCR3+/-;IL-10-/- mice (Fig. 1A and B).

3.2. The severity of colitis was similar between CCR3+/-;IL-10-/- and CCR3-/-;IL-10-/- mice

IL-10-/- mice spontaneously develop colitis in the presence of gut flora, and the severity progresses over time. The analysis was conducted on 15-week-old mice in our facility when sufficiently severe colitis had ensued in IL-10-/- mice. In both CCR3-/-;IL-10-/- and CCR3+/-;IL-10-/- groups, colons showed intestinal wall thickening indicative of the presence of colitis (Fig. 2A); the colonoscopic scores in both groups showed similar levels of pathogenic change compared to control non-colitic mice (Fig. 2B and C). Consistent with these results, the histological evaluation detected no difference in inflammation levels between two groups (Fig. 2D and E).

3.3. Expression levels of inflammatory cytokines were similar between CCR3-/-;IL-10-/- and CCR3+/-;IL-10-/- mice

The data above suggest that the absence of eosinophils due to CCR3 deficiency does not affect the gross colitic phenotype in IL-10-/- mice. However, it does not imply that the immunological profile may not be affected in the absence of eosinophils. To test this point, we looked at the expression levels of inflammatory cytokines in colonic tissue by quantitative RT-PCR. TNF-α—a major pro-inflammatory cytokine known to be upregulated in IL-10-/- mice [23]—was found to be induced in both CCR3-/-;IL-10-/- and CCR3+/-;IL-10-/- groups at similar levels that were significantly higher than that in the control non-colitic group (Fig. 3A). Previous studies have shown that T helper 1 and T helper 17 cells (Th1 and Th17 cells, respectively) undergo expansion in the IL-10-/- model [20, 24]. Additionally, IL-12p40 has been shown to be an essential component of both IL-12 and IL-23 cytokines, which are involved in Th1 differentiation and Th17 expansion, respectively [14]. As predicted, our analysis of IL-12p40 expression revealed similar levels of upregulation in both CCR3-/-;IL-10-/- and CCR3+/-;IL-10-/- groups (Fig. 3B). We also tested IL-4, which is expressed mainly by eosinophils, expecting lower expression levels in CCR3-/-;IL-10-/- than in CCR3+/-;IL-10-/- mice. However, no upregulation of IL-4 was detectable in all mice groups including the non-colitic control, CCR3-/-;IL-10-/-, and CCR3+/-;IL-10-/- mice (data not shown). This suggests that eosinophils are present in the colonic mucosa of IL-10-/- mice due to basal recruitment; they are not, however, activated to produce IL-4, at least at levels above the detection limit of the assay, to confer a difference in the degree of inflammation.

3.4. No differences in Th1 and Th17 expansion between CCR3+/-;IL-10-/- and CCR3-/-;IL-10-/- mice

Finally, we examined if the expansion of Th1 and Th17 cells are affected in the absence of eosinophils in IL-10-/- colitis. Similar levels of hypertrophies of mesenteric lymph nodes

(MLN) were seen in both CCR3-/-;IL-10-/- and CCR3+/-;IL-10-/- mice (Fig. 4A). MLN cells were stimulated with anti-CD3 antibody and cytokines secreted from them were measured by ELISA. The results revealed that both IFN-γ, a Th1-associated cytokine, and IL-17A, a Th17-associated cytokine, were increased in IL-10-/- mice, although no differences were detected in the levels of these cytokines in CCR3-/-;IL-10-/- and CCR3+/-;IL-10-/- mice (Fig. 4B and C). Overall, the data, including that of IL-12p40 expression, indicate that the T cell response in the MLN cells was not affected by the absence of eosinophils.

4. Discussion

Eosinophils constitute a part of the innate immune system and can induce inflammation resulting in tissue damage [25, 26].

We investigated whether the elimination of eosinophils from the intestinal lamina propria can modify the course of colitis in the IL-10 deficient mouse model. We, however, detected no obvious change in both the immunopathology and immunological status of colitic mice tested in our setting.

As mentioned earlier, several studies have indicated that eosinophils, which can produce Th2-type cytokines such as IL-4, IL-5, and IL-13, are detected in the intestinal tissue of IBD patients and that Th2-type cytokines are increased in UC patients. It has also been shown previously that IL-4 deficiency blocked the induction of colitis in IL-10-/- mice [1]. In this specific report, the authors observed significant eosinophilia in the mucosa of IL-10-/- mice but not IL-4-/- IL-10-/- double deficient mice; their studies demonstrated the requirement of IL-4 for the development of colitis and suggested a role for eosinophils in the immunopathology of colitis. This study also used mice of the same C57BL/6 background as was used in our study. There have also been reports that are indicative that IL-4 and eosinophils can promote colitis in other models such as those of dextran sodium sulfate colitis and T cell transfer colitis [27-30]. Griseri et al. showed that eosinophils accumulated in the

colitic tissue in response to GM-CSF and IL-5. Anti-Siglec-F–mediated depletion of eosinophils, but not neutrophils, was found to ameliorate colitis, clearly revealing the importance of eosinophils in chronic colitis. The discrepancy between their observation and ours may be accounted for simply by the difference in the murine model used in the study of dextran sodium sulfate colitis and T cell transfer colitis. The difference in the results of the other study using the IL-10 deficient colitis model and those of ours could be due to differences in the intestinal environment of .It is widely known that the levels of colitis in IL-10 deficient mice are influenced by the husbandry conditions from facility to facility, presumably due to the differences in gut flora. *Helicobacter hepaticus* is a well-known gut dwelling bacterial species capable of inducing colitis in an IL-10 deficient condition [31]; IL-10 deficiency, on the other hand, does not lead to colitis when mice are reared in a germ-free condition [19]. We did not detect upregulation of IL-4, as evaluated by quantitative PCR, in our mice (data not shown). It has been shown that mononuclear phagocytes like macrophages and/or dendritic cells have an important role in the induction of colitis in IL-10-/- mice [20]. Our data indicate that eosinophils are not critically involved in the induction of colitis in IL-10-/- mice; there is a possibility, however, that certain microorganisms that can induce colitis through eosinophil activation in IL-10-/- mice were simply absent in our facility.

There are many therapeutic strategies for human IBD. Progress in immunological research has led to the approval of several new biological reagents for the treatment of IBD including anti-TNF-α antibody, anti-α4β7 blocker, and anti-IL-23 antibody which are all known to function with high efficacy [32-35]. Even with such great advancements, a continued effort to develop new treatments is needed due to the heterogeneous response of patients to different methods of treatment. There are always groups of patients who are unresponsive to treatment and end up with unwanted outcomes such as operations. IBD is a multifactorial disease [36] and disease onset is thought to be triggered by several potential etiologies. We did not detect

an obvious phenotype associated with the loss of CCR3 in the IL-10-deficient colitis model; this does not, however, mean that CCR3 and eosinophils play no role in the colon. The important role played by eosinophils in allergic responses is testimony for the requirement of further research using other colitis models, such as hapten-induced colitis, to see if the pathology is modified by the absence of CCR3 and eosinophils.

In summary, our data indicate that CCR3 deficiency does not change the course of colitis in IL-10 deficient mice tested in our settings. This indicates conditions where eosinophils are possibly quiescent but present in the inflamed colonic tissue without modifying the course of inflammation.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

We thank the animal experiment center of the medical school of Kobe University for the animal care and technical support. We would also like to thank to our deceased former professor, Dr. Takeshi Azuma, for his support. This study was in part supported by the AstraZenecaKK Science promotion grant.

References

[1] S. Specht, S. Arriens, A. Hoerauf, Induction of chronic colitis in IL-10 deficient mice requires IL-4, Microbes Infect, 8 (2006) 694-703.

[2] Y. Jung, M.E. Rothenberg, Roles and regulation of gastrointestinal eosinophils in immunity and disease, J Immunol, 193 (2014) 999-1005.

[3] R.J.L. Azevedo, J. Pinto, H. Ribeiro, et al., Eosinophilic Enterocolitis: An Exceedingly Rare Entity, GE Port J Gastroenterol, 2018;25:184–188 (2018).

[4] S.C. Ng, Inflammatory bowel disease in Asia, Gastroenterol Hepatol (N Y), 9 (2013) 28.

[5] M. Kato, G.M. Kephart, N.J. Talley, et al., Eosinophil infiltration and degranulation in normal human tissue, Anat Rec A Discov Mol Cell Evol Biol, 252 (1998) 418-425.

[6] C.W. DeBrosse, J.W. Case, P.E. Putnam, et al., Quantity and distribution of eosinophils in the gastrointestinal tract of children, Pediatr Dev Pathol, 9 (2006) 210-218.

[7] A. Moon, R.E. Kleinman, Allergic gastroenteropathy in children, Ann Allergy Asthma Immunol, 74 (1995) 5-12; quiz 12-16.

[8] N.J. Talley, M.M. Walker, The Rise and Rise of Eosinophilic Gut Diseases Including Eosinophilic Esophagitis Is Probably Not Explained by the Disappearance of Helicobacter pylori, so Who or What's to Blame?, Am J Gastroenterol, 2018, pp. 941–944.

[9] R. Walsh, T. Gaginella, The eosinophil in inflammatory bowel disease, Scand J Gastroenterol, 26 (1991) 1217-1224.

[10] A.A. Rached, W. El Hajj, Eosinophilic gastroenteritis: Approach to diagnosis and management, World J Gastrointest Pharmacol Ther, 7 (2016) 513.

[11] C.M. Smyth, N. Akasheh, S. Woods, et al., Activated eosinophils in association with enteric nerves in inflammatory bowel disease, PLoS One, 8 (2013) e64216.

[12] F. Torrente, A. Barabino, T. Bellini, et al., Intraepithelial lymphocyte eotaxin-2 expression and perineural mast cell degranulation differentiate allergic/eosinophilic colitis from classic IBD, J Pediatr Gastroenterol Nutr, 59 (2014) 300-307.

[13] F. Heller, I.J. Fuss, E.E. Nieuwenhuis, et al., Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells, Immunity, 17 (2002) 629-638.

[14] W. Strober, I.J. Fuss, Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases, Gastroenterology, 140 (2011) 1756-1767. e1751.

[15] M.F. Neurath, Cytokines in inflammatory bowel disease, Nat Rev Immunol, 14 (2014) 329.

[16] E.A. Garcia-Zepeda, M.E. Rothenberg, R.T. Ownbey, et al., Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia, Nat Med, 2 (1996) 449-456.

[17] R.K. Yantiss, Eosinophils in the GI tract: how many is too many and what do they mean?, Mod Pathol, 28 (2015) S7.

[18] A.A. Humbles, B. Lu, D.S. Friend, et al., The murine CCR3 receptor regulates both the role of eosinophils and mast cells in allergen-induced airway inflammation and hyperresponsiveness, Proc Natl Acad Sci U S A, 99 (2002) 1479-1484.

[19] R. Kühn, J. Löhler, D. Rennick, et al., Interleukin-10-deficient mice develop chronic enterocolitis, Cell J, 75 (1993) 263-274.

[20] N. Hoshi, D. Schenten, S.A. Nish, et al., MyD88 signalling in colonic mononuclear phagocytes drives colitis in IL-10-deficient mice, Nat Commun, 3 (2012) 1120.

[21] C.-Y. Chiu, B. Gomolka, C. Dierkes, et al., Omega-6 docosapentaenoic acid-derived resolvins and 17-hydroxydocosahexaenoic acid modulate macrophage function and alleviate experimental colitis, Inflamm Res, 61 (2012) 967-976.

[22] S. Adachi, N. Hoshi, J. Inoue, et al., Indigo Naturalis Ameliorates Oxazolone-Induced Dermatitis but Aggravates Colitis by Changing the Composition of Gut Microflora, Int Arch Allergy Immunol, 173 (2017) 23-33.

[23] E. Talero, A. Alcaide, J. Ávila-Román, et al., Expression patterns of sirtuin 1-AMPK-autophagy pathway in chronic colitis and inflammation-associated colon neoplasia in IL-10-deficient mice, Int Immunopharmacol, 35 (2016) 248-256.

[24] T.D. Yan, D. Black, R. Savady, et al., Systematic review on the efficacy of cytoreductive surgery combined with perioperative intraperitoneal chemotherapy for peritoneal carcinomatosis from colorectal carcinoma, J Clin Oncol, 24 (2006) 4011-4019.

[25] A.A. Alfadda, M.A. Storr, E.A. Shaffer, Eosinophilic colitis: epidemiology, clinical features, and current management, Therap Adv Gastroenterol, 4 (2011) 301-309.

[26] E.T. Cherian, H.C. Zhang, K.B. Guttenberg, et al., Looking Beyond the Obvious: Eosinophilic Enterocolitis, Am J Med, 131 (2018) e227-e229.

[27] R. Hadar, M. Itay, I. Michal, et al., Transcriptome profiling of mouse colonic eosinophils reveals a key role for eosinophils in the induction of s100a8 and s100a9 in mucosal healing, Sci Rep, 7 (2017) 7117.

[28] T. Griseri, I.C. Arnold, C. Pearson, et al., Granulocyte macrophage colony-stimulating factor-activated eosinophils promote interleukin-23 driven chronic colitis, Immunity, 43 (2015) 187-199.

[29] A. Waddell, R. Ahrens, Y.-T. Tsai, et al., Intestinal CCL11 and Eosinophilic Inflammation Is Regulated by Myeloid Cell–Specific RelA/p65 in Mice, J Immunol., (2013) 1200057.

[30] M.M. Fort, J. Cheung, D. Yen, et al., IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo, Immunity, 15 (2001) 985-995.

[31] M.C. Kullberg, A.G. Rothfuchs, D. Jankovic, et al., Helicobacter hepaticus-induced colitis in interleukin-10-deficient mice: cytokine requirements for the induction and maintenance of intestinal inflammation, Infect Immun, 69 (2001) 4232-4241.

[32] G. Van Assche, M. Van Ranst, R. Sciot, et al., Progressive multifocal leukoencephalopathy after natalizumab therapy for Crohn's disease, N Engl J Med, 353 (2005) 362-368.

[33] W. Reinisch, W.J. Sandborn, D.W. Hommes, et al., Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial, Gut, 60 (2011) 780-787.

[34] B.G. Feagan, P. Rutgeerts, B.E. Sands, et al., Vedolizumab as induction and maintenance therapy for ulcerative colitis, N Engl J Med, 369 (2013) 699-710.

[35] B.G. Feagan, W.J. Sandborn, C. Gasink, et al., Ustekinumab as induction and maintenance therapy for Crohn's disease, N Engl J Med, 375 (2016) 1946-1960.

[36] K.L. Wallace, L.-B. Zheng, Y. Kanazawa, et al., Immunopathology of inflammatory bowel disease, World J Gastroenterol, 20 (2014) 6.

Figure legends:

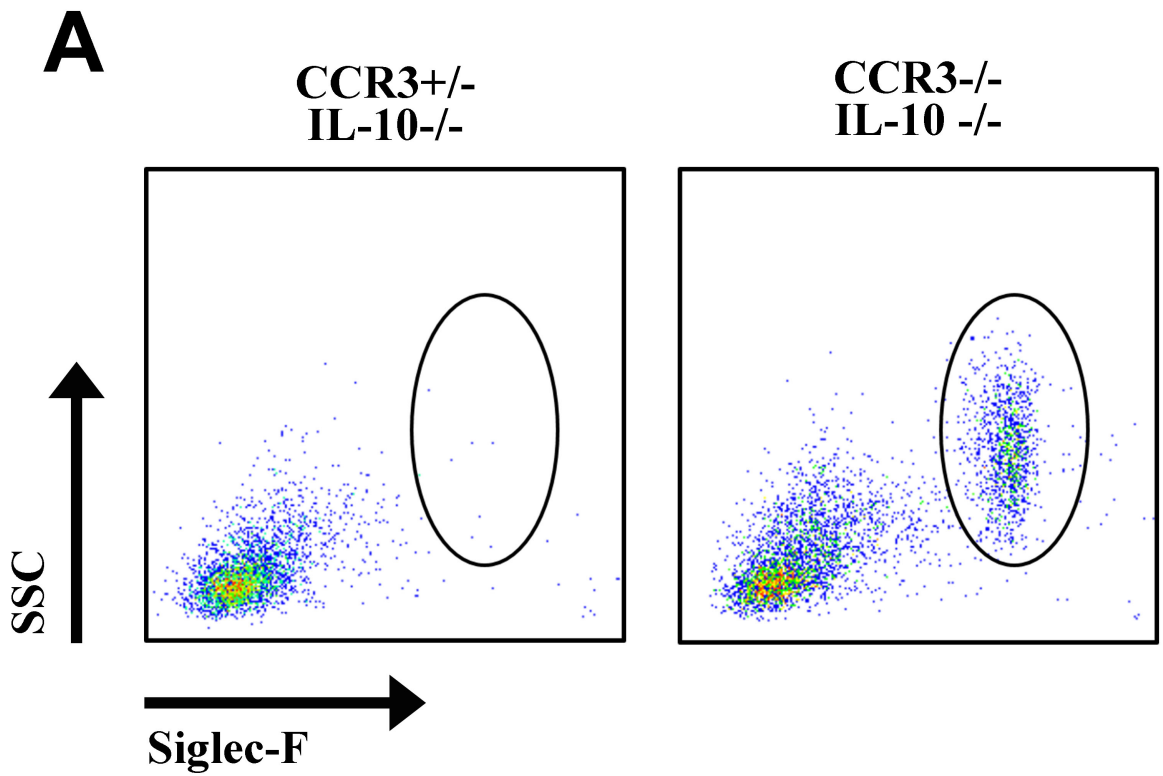
Fig. 1. FACS analysis of eosinophils in colonic lamina propria. CCR3+/-;IL-10-/- (n = 4) : Fifteen-week-old CCR3-/-;IL-10-/- (n = 3) mice were sacrificed and lamina propria cells were isolated from mouse colons. (A) Representatives of FACS analysis of colonic lamina propria cells: Siglec-F^+ cells gated on $CD11c^-$ $CD11b^+$ cells. (B) The percentage of Siglec-F^+ cells in $CD11c^-$ $CD11b^+$ cells. Error bars represent ± SEM. * = $p < 0.05$ using the Student's *t*-test.

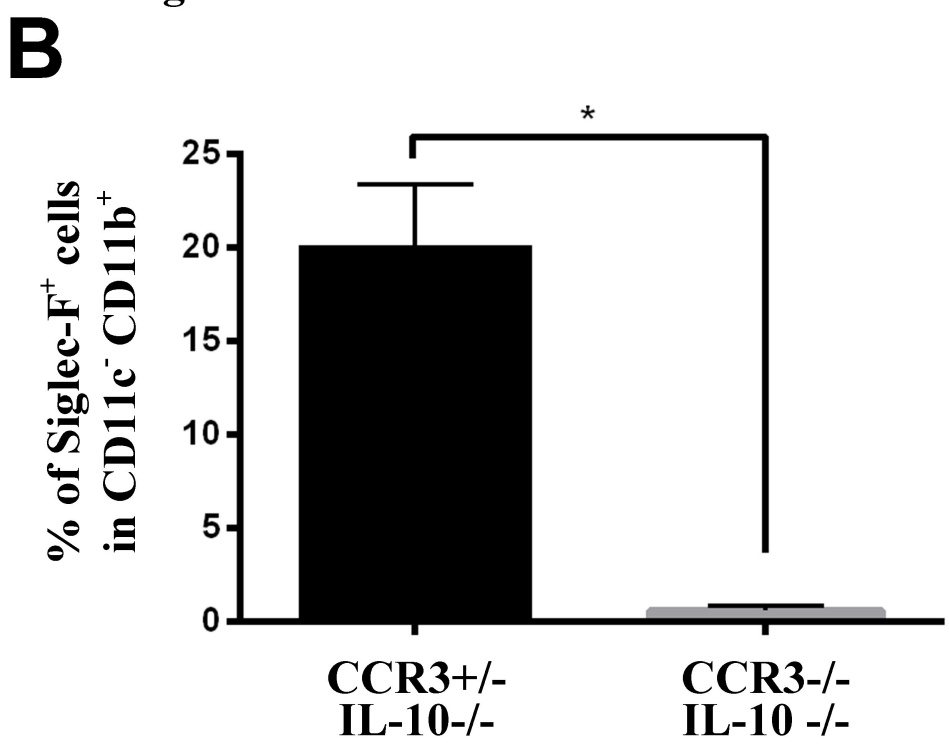
Fig. 2. No difference in severity of colitis between CCR3+/-;IL-10-/- and CCR3-/-;IL-10-/- mice. (A) Photographs of representative colons. (B) Representatives of colonoscopic images, and (C) scoring. (D) H and E staining of representative sections of colons, and (E) histological scoring. Wild type (n = 12); CCR3+/-;IL-10-/- (n = 11); and CCR3-/-;IL-10-/- (n = 8) mice were used at of 15 weeks of age. Error bars represent ± SEM. * = $p < 0.05$ using the Student's *t*-test.

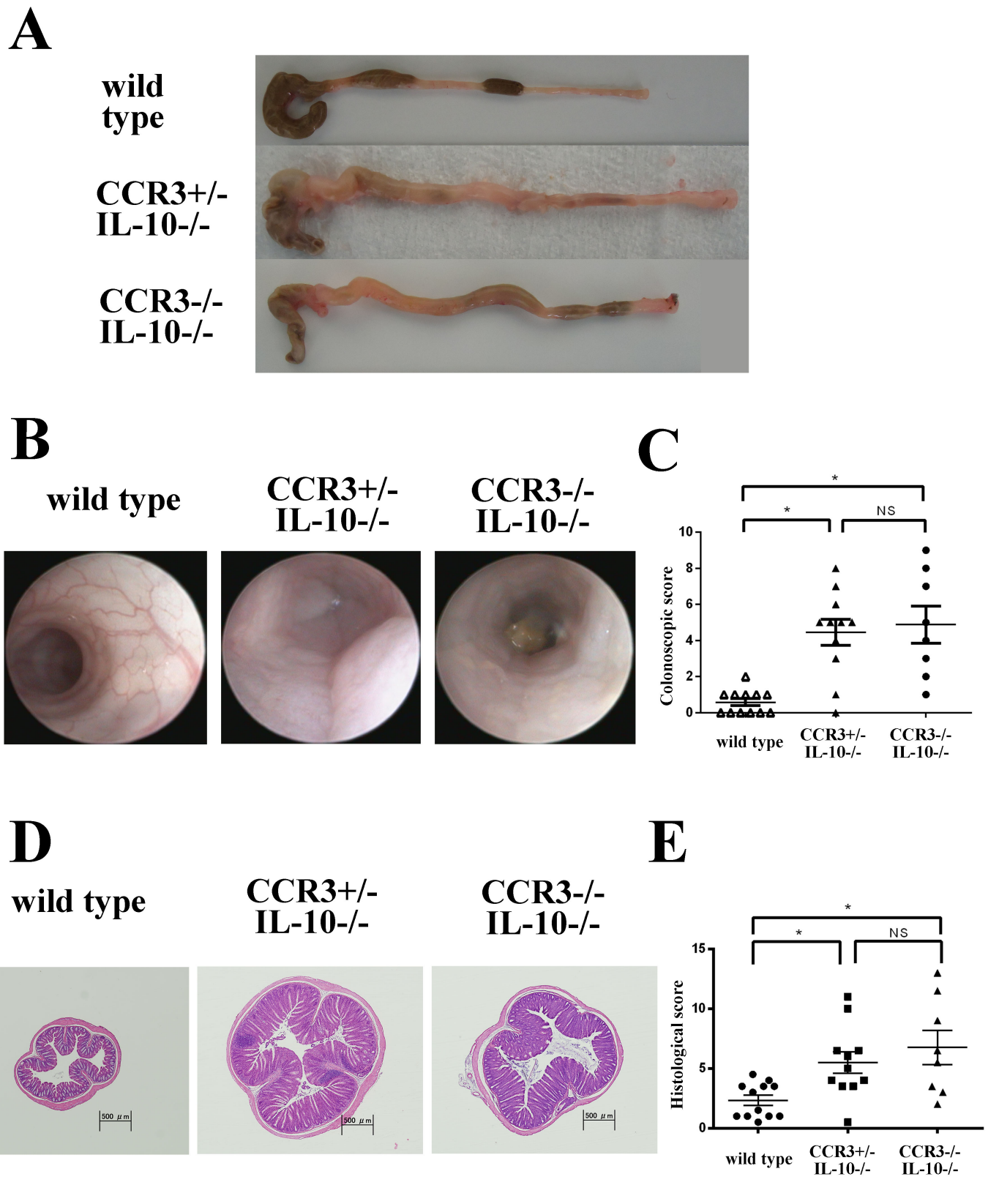
Fig. 3. No difference in expression levels of pro-inflammatory cytokines in colonic tissue between CCR3+/-;IL-10-/- and CCR3-/-;IL-10-/- mice. The expression levels of (A) TNF-α and (B) IL-12p40 in colonic tissue were evaluated by real-time PCR. Wild type (n = 12); CCR3+/-;IL-10-/- (n = 11); CCR3-/-;IL-10-/- (n = 8). Error bars represent ± SEM. * = $p < 0.05$ using the Student's *t*-test.

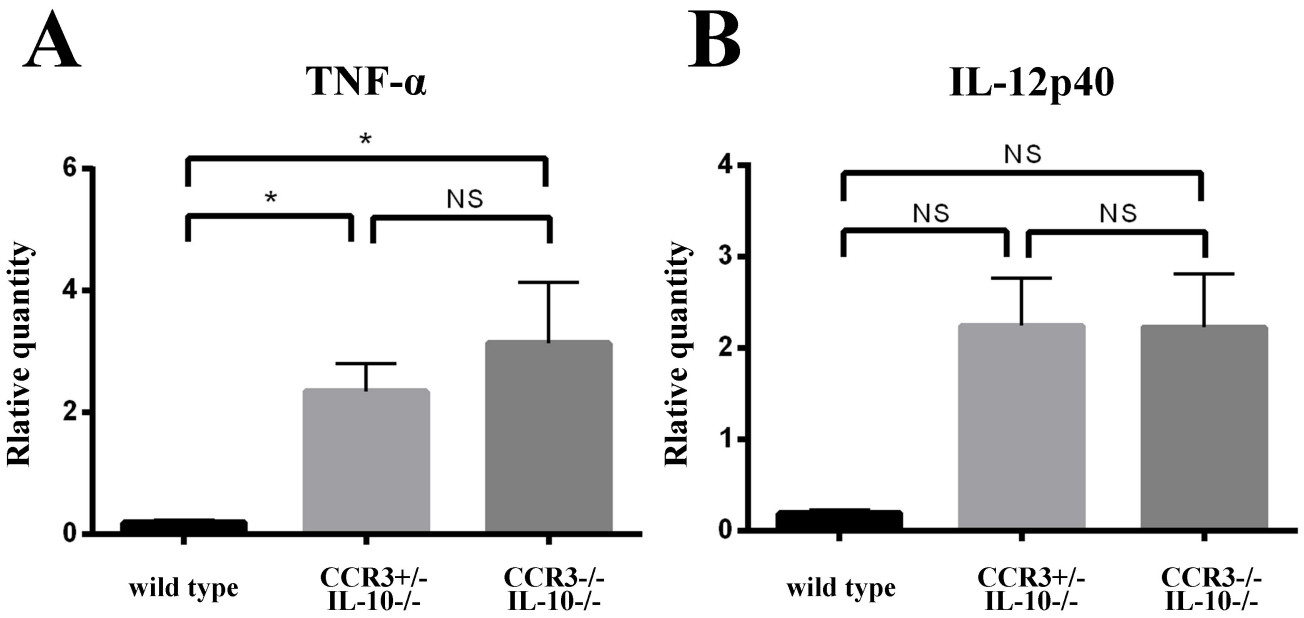
Fig. 4. No difference in cytokine production from Th1 and Th17 cells between CCR3+/-;IL-10-/- and CCR3-/-;IL-10-/- mice. Mesenteric lymph nodes were harvested from 15-week-old mice. (A) Cell counts and (B) IL-17A and (C) IFN-γ measurements from T cells after stimulation with anti-CD3 antibody. Cytokine levels were evaluated by ELISA. Wild type (n

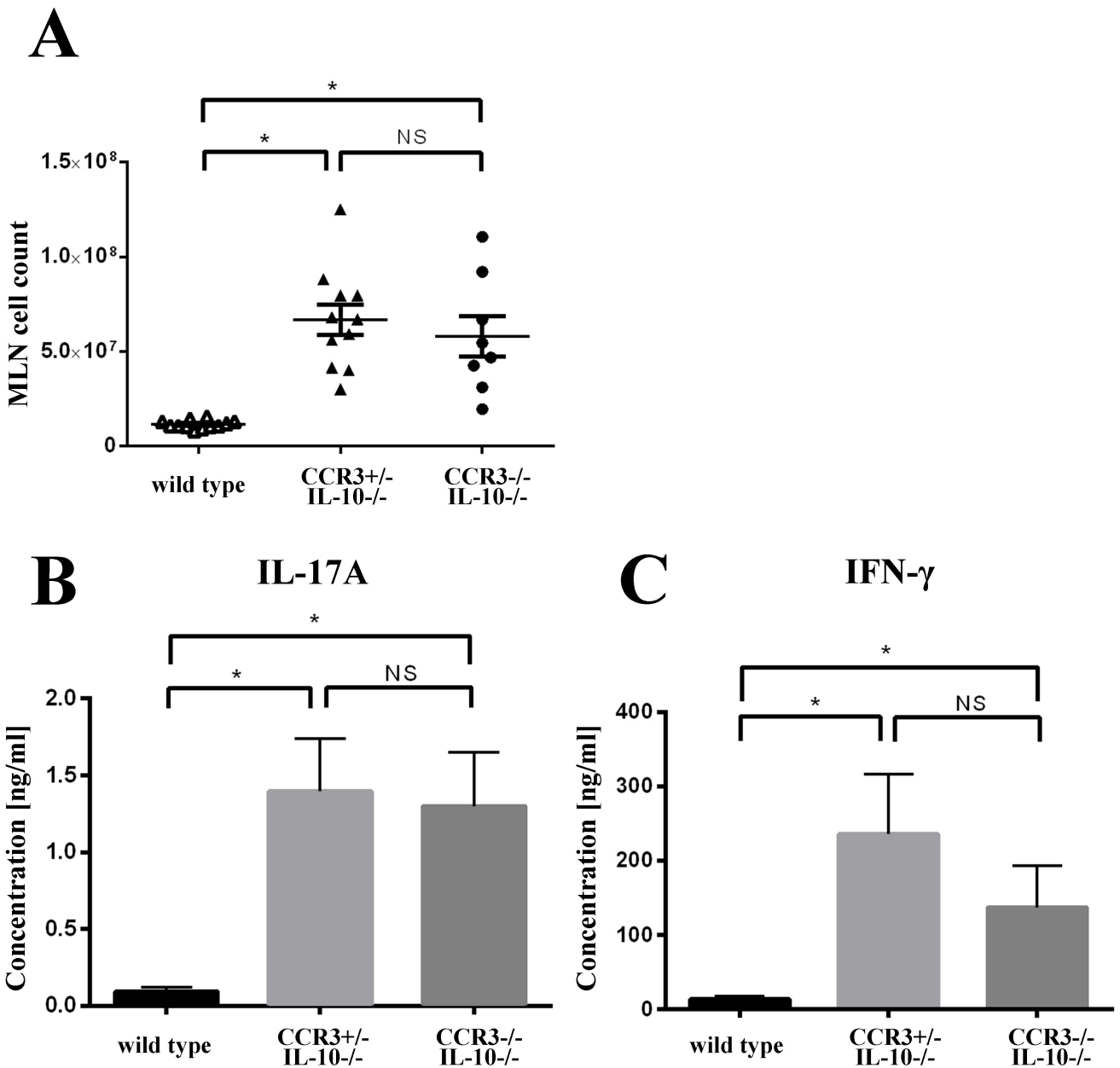
= 12); CCR3+/-;IL-10-/- (n = 11); CCR3-/-;IL-10-/- (n = 8). Error bars represent ± SEM. * = $p < 0.05$ using the Student's *t*-test.


A
CCR3+/-
IL-10-/-
CCR3-/-
IL-10 -/-
SSC
Siglec-F


B
*
25
20
15
10
5
0
% of Siglec-F+ cells
in CD11c- CD11b+
CCR3+/-
IL-10-/-
CCR3-/-
IL-10 -/-


A
wild type
CCR3+/- IL-10-/-
CCR3-/- IL-10-/-
B
wild type
CCR3+/- IL-10-/-
CCR3-/- IL-10-/-
C
Colonoscopic score
0
2
4
6
8
10
*
*
NS
wild type
CCR3+/- IL-10-/-
CCR3-/- IL-10-/-
D
wild type
CCR3+/- IL-10-/-
CCR3-/- IL-10-/-
500 μm
500 μm
500 μm
E
Histological score
0
5
10
15
*
*
NS
wild type
CCR3+/- IL-10-/-
CCR3-/- IL-10-/-


A
TNF-α
Rlative quantity
6
4
2
0
*
*
NS
wild type
CCR3+/-
IL-10-/-
CCR3-/-
IL-10-/-
B
IL-12p40
Rlative quantity
4
3
2
1
0
NS
NS
NS
wild type
CCR3+/-
IL-10-/-
CCR3-/-
IL-10-/-


A
*
*
NS
1.5×10^8
1.0×10^8
5.0×10^7
0
MLN cell count
wild type
CCR3+/-
IL-10-/-
CCR3-/-
IL-10-/-
B
IL-17A
*
*
NS
2.0
1.5
1.0
0.5
0.0
Concentration [ng/ml]
wild type
CCR3+/-
IL-10-/-
CCR3-/-
IL-10-/-
C
IFN-γ
*
*
NS
400
300
200
100
Concentration [ng/ml]
wild type
CCR3+/-
IL-10-/-
CCR3-/-
IL-10-/-