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Pharmacological effects of TAK-828F: an orally available RORγt inverse agonist, in mouse colitis model and human blood cells of inflammatory bowel disease

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Abstract

Objective and design To evaluate the potency of RORγt blockade for treatment of Inflammatory Bowel Disease (IBD), the efficacy of TAK-828F, a novel RORγt inverse agonist, in anti-TNF-α mAb non-responsive mouse colitis model and effect of TAK-828F on IL-17 production in peripheral mononuclear blood cells (PBMCs) of anti-TNF-α naive and treatment-failure patients of IBD was investigated.

Methods and results The colitis model showed Th17-dependent pathogenicity and response to anti-IL-12/23p40 monoclonal antibody (mAb), but no response to anti-TNF-α mAb. In the model, TAK-828F, at oral dosages of 1 and 3 mg/kg, inhibited progression of colitis and reduced the immune reaction that characterize Th17 cells. Anti-IL-17A mAb showed neither efficacy nor change in the T cell population and colonic gene expression in the model. In the normal mouse, a 4-week treatment of TAK-828F at 30 mg/kg did not severely reduce lymphocyte cell counts in peripheral and intestinal mucosa, which was observed in RORγ$^{-/-}$ mice. TAK-828F strongly inhibited IL-17 gene expression with IC_{50} values from 21.4 to 34.4 nmol/L in PBMCs from anti-TNF mAb naive and treatment-failure patients of IBD.

Conclusions These results indicate that RORγt blockade would provide an effective approach for treating refractory patients with IBD by blocking IL-23/Th17 pathway.

Keywords Inflammatory bowel disease · TAK-828F · RORγt inverse agonist · IL-23/Th17 pathway · Mouse colitis model

Introduction

Inflammatory bowel disease (IBD) is a chronic and refractory gastrointestinal disease along with a severe decrease in QOLs [1, 2]. To treat the patients with IBD, anti-inflammatory and immunomodulatory drugs such as 5-aminosalicylates, corticosteroids and azathioprine are used. However, the efficacy of these drugs is restricted in some patients, and long-term use of corticosteroids and azathioprine is limited due to their side-effects [3]. In the patients who show resistance to these agents, treatment with anti-TNF-α monoclonal antibodies (mAb) and vedolizumab are clinically approved [3–5]. Although these biologics are providing a lot of benefit in the treatment of severe IBD, non-responding patients still exist [4]. Additionally, expensive drug prices and periodic hospital visits for administration are also a burden on the patients. Thus, there is a high unmet medical need for a novel oral drug that possesses strong efficacy, a favorable

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safety profile and less expensive alternatives to treat refractory patients to current therapy, especially against biologics.

The IL-23/Th17 axis has been identified to play a major role in the pathogenesis of IBD. For example, genome-wide association studies indicate that interleukin-23 (IL-23) and additional genes in the IL-23/Th17 pathway (IL-12p40, JAK2, STAT3, CCR6 and TNFSF15) are associated with Crohn's disease (CD) and partially to ulcerative colitis (UC) [6–9] Anti-IL-12/23p40 monoclonal antibody (mAb) and anti-IL-23 mAb demonstrated significant efficacy in anti-TNF-α mAb refractory patients with CD [10–13] indicating the large impact of IL-23/Th17 pathway in the pathogenesis of severe CD. Involvement of IL-23 leading to Th17 cell differentiation, expansion and stabilization [14, 15] indicates that blocking IL-23/Th17 axis is an attractive therapeutic strategy to treat patients with severe CD, especially for non-responders to anti-TNF-α mAb. In addition to Th17, the subset of IL-17 and IFN-γ double-producing Th17 (Th1/17) cells has been recently identified, which may also be involved in the pathology of inflammatory diseases including IBD and multiple sclerosis (MS) [16, 17]. RORγt has essential roles for the differentiation of Th17 and Th1/17 cells and activation of these cells [17–19]. RORγt dysregulation is also known to induce immune imbalance by disturbing the equilibrium of pro inflammatory IL-17$^+$ and regulatory IL-10$^+$ T cell populations [20]. Thus, RORγt could be an attractive target for the treatment of Th17 and Th1/17-related several immune diseases.

We have discovered an orally available novel small molecule RORγt inverse agonist, TAK-828F [21]. In a cell-based functional assay, TAK-828F inhibited the transcriptional activity of human RORγt with an IC_{50} of 6.1 nM and TAK-828F showed clear selectivity when tested against 21 other types of nuclear receptors including RORα and RORβ [22]. TAK-828F, at 10 and 100 nM, suppressed IL-17 production, but does not inhibit IFN-γ, from murine splenocytes and healthy human peripheral blood mononuclear cells (PBMC) [23]. TAK-828F also inhibited Th17 cell differentiation, without affecting Th1 cell differentiation, from primary naive T cells of mouse and human [23]. In the in vivo efficacy study, TAK-828F showed protective and therapeutic efficacy in the activated CD4$^+$ T cell transferred colitis model [24]. This colitis model was highly responsive to anti-TNF-α mAb indicating that RORγt blockade may have a potency in anti-TNF-α mAb naive patients with IBD. In addition to anti-TNF-α mAb responding patients, non-responding patients to anti-TNF-α mAb are also clinically important as mentioned above. Therefore, to evaluate whether RORγt blockade has the potential to show efficacy in anti-TNF-α mAb non-responding patients, we have conducted efficacy studies of TAK-828F in the naive T cell transfer colitis model that is non-responding to anti-TNF-α mAb. In addition, efficacy and pharmacological profiles of TAK-828F was directly compared to that of anti-IL-17A mAb, since anti-IL-17A mAb failed in a clinical trial of CD [25]. Additionally, we have investigated the effects of TAK-828F on the immune cell population in normal mouse to evaluate the impact of RORγt inhibition on immune status.

We previously reported the potent inhibitory effect of TAK-828F on IL-17 production from in vitro cultured PBMCs of healthy human volunteers [23]. To discuss more deeply the human relevancy of outcome from animal models described above, we established ex vivo Th-17-related gene expression model by use of PBMC isolated from IBD patients of anti-TNF-α mAb naive and failure, and inhibitory effects of TAK-828F in this model were investigated. To evaluate the contribution of RORγt-pathway in the pathogenicity of IBD, plasma level of IL-17A was also measured. We have discussed the clinical benefit of RORγt blockade for the therapeutic option of IBD not only on the basis of efficacy studies in mouse colitis model but also on the data obtained by use of human PBMCs derived from IBD patients of both anti-TNF-α mAb naive and failure.

Materials and methods

Animals

Balb/c mice (female) were purchased from Charles River Japan (Kanagawa, Japan). C.B-17/Icr-scid mice (SCID mice, female) were purchased from CLEA Japan. (Tokyo, Japan). C.B-17/Icr-scid mice were used as recipient mice and bred individually on white chip (Paperclean, Japan SLC, Shizuoka, Japan). RORγ knockout (RORγ$^{-/-}$) mice were generated and bred at Takeda Pharmaceutical Company, Ltd. (Tokyo, Japan). Mice were maintained under specific pathogen-free conditions. All procedures were performed in accordance with the standards for humane care, and treatment of research animals was approved by IACUC (Institutional Animal Care and Use Committee) in Takeda Pharmaceutical Company, Ltd. (Approval no. 10797, 10916).

Generation of RORγ$^{-/-}$ mice

RORγ$^{-/-}$ mice were newly generated at Takeda Pharmaceutical Company, Ltd. (Japan, Uga et al. in submission). The targeting vector pKO-BAC-RORγ was constructed by Red/ET recombination system (GeneBridges, Heidelberg, Germany) to modify BAC with mouse RORγ gene (RP23-263K17; Advanced GenoTechs, Ibaraki, Japan). pKO-BAC-RORγ was designed to flox exon 3 and 4, containing the PGK-Neo cassette. pKO-BAC-RORγ was linearized by AscI and electroporated into Balb/cA embryonic stem cells (ESCs). Clones with a disrupted RORγ allele were screened after G418 selection. Exon 3 and 4 were removed by transient

expression of pCAG-Cre plasmids in recombinant ESCs. Chimeric mice were generated by tetraploid complementation method described previously [26]. The chimeric mice were crossed with Balb/cA mice to obtain mice heterozygous for the mutant allele. Heterozygous mice were intercrossed to obtain animals homozygous for the mutant allele and to obtain wild-type littermate controls. RORγ gene consists of two isomers of RORγ and RORγt [27]. DNA binding domain, ligand binding domain and activation function 2 are identical to each other, and only N-terminal 19 amino acid residues lack in RORγt [27]. Thus, deletion of RORγ gene results in the disruption of RORγt gene of the mice.

Chemicals

TAK-828F was synthesized at Takeda Pharmaceutical Company, Ltd. (Japan).

Experimental colitis and treatment of TAK-828F and neutralizing antibody

Mouse colitis was induced by adoptive transferring of naive T cells of Balb/c mice to SCID mice [23]. Briefly, $CD4^+CD62L^+$ naive T cells (2×10^5 cells/mouse) from Balb/c mice were intravenously injected into SCID mice (day 0). In the experiment with $ROR\gamma^{-/-}$ mice, naive T cells (2×10^5 cells/mouse) from $ROR\gamma^{-/-}$ and WT mice were intravenously injected into SCID mice. On day 21 after T cell transfer, diarrhea score for stool consistency was graded under blind fashion and mice were sacrificed under anesthesia. The colon of each mouse was surgically removed, rinsed with saline, and the weight of the colon was measured. Mesenteric lymph node (MLN) of each mouse was also collected for flow cytometry analysis.

TAK-828F was suspended in 0.5% methyl cellulose (0.5% MC) and administered to the mice via oral gavage. The test compound was administered once a day on day 0, and twice a day from days 1 to 20. On day 21, diarrhea score for stool consistency and colon weight of each mouse was measured under blind fashion [23].

Anti-TNF-α mAb (clone: XT3.11, Bio X Cell, West Lebanon, NH) and its isotype Ig G1 (clone: MOPC21, Bio X Cell) was intraperitoneally (i.p.) administered at a dose of 0.1 mg/mouse on days 0, 5, 9, 13 and 17 after the T cell transfer since anti-TNF-α mAb showed strong protective efficacy under these conditions in the activated T cell transfer mouse colitis model [28]. In the preliminary study, the neutralization activity of the anti-TNF-α mAb was measured in TNF-α induced cytotoxicity against L-929 cells and showed nearly complete inhibition at more than 3 μg/mL (data not shown). In addition, plasma level of the anti-TNF-α mAb was maintained over 30 μg/mL for 5 days in the mouse plasma after single intraperitoneal administration of the mAb (data not shown). These facts indicate that sufficient amount of anti- TNF-α mAb to neutralize TNF-α in the mouse plasma was administered in the efficacy study. Anti-IL-12/23p40 mAb (clone: C17.8, R&D, Minneapolis, MN) and its isotype IgG2 (clone: 54447, R&D) were also administered at 0.1 mg/mouse with the same administration schedule with anti-TNF-α mAb, since this mAb also showed strong efficacy in the activated T cell transfer colitis model under this condition (data not shown). Anti-IL-17A mAb (clone: 17A3, Bio X cell) and its isotype Ig G1 (clone: MOPC21, Bio X Cell) were used in the efficacy study. Anti-IL-17A mAb (clone: 17A3, Bio X Cell) at 1 and 10 μg/mL almost completely inhibited IL-17-induced IL-6 production from NIH 3T3 cells in vitro, showing a strong neutralizing effect (data not shown). A plasma level of > 10 μg/mL was sustained for 7 days after single intraperitoneal administration of 0.02 mg/mouse of anti-IL-17A mAb in normal mice. To keep high plasma concentration (> 10 μg/mL) of anti-IL-17A mAb in the plasma of mice, anti-IL-17A mAb at a dose of 0.02 mg/mouse was i.p. administered on days 0, 7 and 14 in the experiment. With this dose administration schedule, anti-IL-17A mAb showed significant efficacy in experimental autoimmune encephalomyelitis model of mice (data not shown).

Intracellular cytokine staining

Single cell suspensions prepared from MLNs were stimulated with PMA (50 ng/mL, Wako Pure Chemical Industries, Ltd, Osaka, Japan) and ionomycin (1 μg/mL, Wako Pure Chemical Industries, Ltd), in the presence of transport inhibitor containing monensin (BD Biosciences, Franklin Lakes, NJ) for 4 h in RPMI-1640 medium containing 10% of FBS. After blocking Fc receptor by anti-CD16/CD32 mAb (clone: 2.4G2, Bio X Cell), cells were stained with PE-conjugated anti-CD4 mAb (BioLegend, San Diego, CA). Stained cells were fixed and permeabilized with Fixation/Permeabilization solution (BD Biosciences). The intracellular cytokine staining was carried out using FITC-conjugated anti-IFN-γ mAb and Alexa Fluor 647-conjugated anti-IL-17A mAb (BioLegend). Flow cytometry analysis was performed using BD Accuri C6 Flow Cytometer (BD Biosciences). The population of Th17, Th1/17 and Th1 cells were defined as follows: Th17, IL-17^+ IFN-γ^- cells gated on $CD4^+$ cells; Th1/17, IL-17^+ IFN-γ^+ cells gated on $CD4^+$ cells; Th1, IL-17^- IFN-γ^+ cells gated on $CD4^+$ cells.

Real-time quantitative RT-PCR

Colon was stored in RNA later (Qiagen, Hilden, Germany) at 4 °C. Total RNA was isolated using RNeasy Mini Kit (Qiagen) and DNaseI (Qiagen) to avoid genomic DNA contamination, according to the manufacturer's instructions.

High Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, CA) was used for cDNA synthesis. Quantitative PCR reactions were performed on a ViiA 7 Real-Time PCR System (Life Technologies), using TaqMan Fast Advanced Master Mix (Life Technologies) with specific primers on TaqMan Gene Expression Assays (Life Technologies) according to the manufacturer's manual. FAM-probed primers with following assay identification numbers were used: *Il17a*, Mm00439618_m1; *Il17f*, Mm00521423_m1; *Il22*, Mm01226722_g1; *Ifng*, Mm00801778_m1; *Il10*, Mm00439614_m1. The data were normalized to β-actin (*Actb*) gene expression.

Histopathology

Two small pieces from proximal and distal portions of the colon were dissected from each mouse and placed in 10 vol% neutral buffered formalin. All tissues were embedded in paraffin, sectioned in a cross-sectional manner and stained with hematoxylin and eosin. Histopathological evaluation was performed independently by two pathologists, and the following criteria were used.

Histopathological scoring system

	Score	Grade	Criteria
Mucosal regeneration/hyperplasia	0	None	Not remarkable
	1	Minimal	Regeneration/hyperplasia of mucosa without goblet cells is observed in focal area
	2	Mild	Multifocal regeneration/hyperplasia of mucosa are observed in about half of the mucosal area
	3	Moderate	Regeneration/hyperplasia of mucosa is observed in about half to almost all the mucosal area with slightly increased mucosal thickness. Goblet cells are observed focally
	4	Marked	Regeneration/hyperplasia of mucosa is observed diffusely with twofold mucosal thickness. Goblet cells are scant
Mononuclear cell infiltration	0	None	Not remarkable
	1	Minimal	Focal or multifocal infiltration of mononuclear cells (mainly lymphocytes) in mucosa and/or submucosa
	2	Mild	Diffuse infiltration of mononuclear cells (mainly lymphocytes in mucosal and/or submucosa
Inflammatory cell infiltration	0	None	Not remarkable
	1	Minimal	Focal infiltrations of inflammatory cells (mainly neutrophils) in mucosa
Crypt abscesses/cell debris in lumen	0	None	Not remarkable
	1	Minimal	Abscess and/or cell debris in the lumen of the crypts

Histopathological scores of all findings from both proximal and distal sections of the colon were combined to calculate the total histopathological score for each animal (maximum score 16).

Analysis of immune cell population

TAK-828F was suspended in 0.5% methyl cellulose (0.5% MC) and orally administered to the normal Balb/c mice for 28 days (3 or 30 mg/kg, b.i.d.). TAK-828F-treated mice and $ROR\gamma^{-/-}$ mice (9 weeks) were sacrificed, spleen and small intestine were collected. Small intestine was used in the assay, since innate lymphoid 3 (ILC3) cells were mainly localized in the small intestine, but not in the colon, of the normal mice in our preliminary assay (data not shown). Spleen cells were used as peripheral cells in the assay, since mouse blood cells are unstable and therefore difficult to accurately measure cell count by flow cytometry analysis. Total lymphoid cells were recovered from murine splenocytes by lympholyte-M (Cedarlane Laboratories, Burlington, Canada). Then cell suspension was treated with HLB solution (IBL, Gunma, Japan) to hemolyze. Mucosal lymphocytes of small intestine were collected using Lamina Propria Dissociation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. For intracellular cytokine staining, single cell suspensions were stimulated with PMA (50 ng/mL, Wako Pure Chemical Industries, Ltd) and ionomycin (1 μg/mL, Wako Pure Chemical Industries, Ltd), in the presence of transport inhibitor containing monensin (BD Biosciences) for 4 h in RPMI-1640 medium containing 10% of FBS. For blocking Fc receptor anti-CD16/CD32 mAb (BD Biosciences) was used. For staining each cell surface makers, PE-conjugated

anti-mouse NKp46 mAb, PE conjugated anti-mouse CD4, PerCP/Cy5.5 conjugated anti-mouse CD3 (BioLegend), V450 conjugated anti-mouse DX5, V450 conjugated anti-Mouse CD45R (BD Biosciences), PerCP-Cy5.5 conjugated anti-mouse/human CD44 and FITC conjugated anti-mouse CD8a (Affymetrix, Santa Clara, CA) were used. For intracellular staining, after staining with antibodies against cell surface markers, cell suspensions were fixed and permeabilized with Foxp3/Transcription Factor Staining Buffer Set (Affymetrix). Antibodies for intracellular staining were FITC conjugated anti-mouse IFN-γ, Alexa Fluor 647 conjugated anti-mouse IL-17A (BioLegend), Alexa Fluor 647 conjugated anti-mouse RORγt (BD Biosciences) and FITC conjugated anti-mouse/rat Foxp3 (Affymetrix). Flow cytometry analysis was performed using BD FACSCanto II Flow Cytometer (BD Biosciences). The population of total $CD4^+$ T, naive $CD4^+$ T, memory $CD4^+$ T, activated $CD4^+$ T, $CD8^+$ T, B, NK, naturally occurring regulatory T cell (nTreg), Th1, Th17, innate lymphoid 3 (ILC3), natural cytotoxicity receptors $(NCR)^+$ ILC3 and NCR^- ILC3 cells were defined as follows: total $CD4^+$ T cells, $CD4^+$ cells; naive $CD4^+$ T cells, $CD25^-CD44^-$ cells gated on $CD4^+$ cells; memory $CD4^+$ T cells, $CD25^-CD44^+$ cells gated on $CD4^+$ cells; activated $CD4^+$ T cells, $CD25^+CD44^+$ cells gated on $CD4^+$ cells; $CD8^+$ T cells, $CD8^+$ cells; B cells, $B220^+$ cells; NK cells, $CD3^-DX5^+$ cells; nTreg, $CD25^+Foxp3^+$ cells gated on $CD4^+$ cells; Th1, IFN-γ^+IL-17^- cells gated on $CD4^+$ cells; Th17, IFN-γ^-IL-17^+ cells gated on $CD4^+$ cells; ILC3, $CD3^-$RORγt$^+$ cells; NCR^+ ILC3, $NKp46^+$ cells gated on ILC3; NCR^- ILC3, $NKp46^-$ cells gated on ILC3.

In vitro assay by use of blood samples from IBD patients

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethics committees of both Keio University School of Medicine and Takeda Pharmaceutical Company Ltd. Signed informed consent forms were obtained from all patients and volunteers, and all the data were analyzed anonymously throughout the study. The diagnosis of ulcerative colitis (UC) and Crohn's disease (CD) was based on established clinical, radiographic, endoscopic and histopathological criteria. Patients' characteristics were determined from medical records and interviews. Thirteen UC patients, twelve CD patients, and five healthy volunteers were recruited from Keio University Hospital. The clinical disease activity in the patients with UC and CD was determined by the diagnostic guidelines of the Ministry of Health, Labor and Welfare in Japan. Active UC was defined as partial Mayo score ≥ 3, and active CD was defined as Crohn's Disease Activity Index > 150. The inclusion criteria to participate in this study were as follows; age over 16 years and patients with active UC who were steroid-dependent/resistant or patients with active CD. Blood samples were collected from patients who have just started anti-TNF-α mAb treatments (anti-TNF naive) immediately before the first infusion, or ones whose anti-TNF-α mAb treatments have just failed (anti-TNF failure) immediately before dose escalation or switching to other therapies, with use of heparin as an anticoagulant. When anti-TNF naive patients were diagnosed as an anti-TNF failure within 16 weeks since started the treatments, their blood samples were collected again and used as those from anti-TNF failure. As a control, blood samples from five healthy volunteers were also collected.

PBMCs were isolated from fresh blood by using Ficoll-paque density gradient centrifugation at $400\times g$ for 40 min at 4 °C. The PBMCs were seeded on flat 96 well plates at 160,000 cells/well in RPMI1640 (Gibco, Co Dublin, Ireland) supplemented with 10% FBS and 1 × Penicillin–Streptomycin (Gibco), and then TAK-828F was added to each well at indicated final concentrations. Thirty minutes after incubation at room temperature, Dynabeads™ Human T-Activator CD3/CD28 (Gibco) conditioned in the same culture medium were added to each well at 160,000 beads/well. These plates were further incubated at 37 °C in 5% CO_2 for 20 h, and then total RNA of each well was isolated by RNeasy Plus Micro Kit (Qiagen) according to the manufacturer's instructions. The extracted RNA was then reverse transcribed into cDNA by using High Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA) and quantified by real-time PCR analysis using EXPRESS qPCR Supermix (Invitrogen, Carlsbad, CA). Following TaqMan primers and probe sets purchased from Applied Biosystems were used: human IL17a (Hs00174383_m1) and RPLP0 (4333761T). Thermal cycle reactions were performed on StepOnePlus Real-Time PCR System (Applied Biosystems) or 7900HT FAST Real-Time PCR System (Applied Biosystems). The data were analyzed by normalizing Ct values of IL17A to those of the housekeeping gene RPLP0.

For cytokine assays, plasma samples were prepared by centrifugation at 3000 rpm at 4 °C for 15 min and then stored at − 80 °C until analysis. Concentrations of IL17a in plasma were measured using Human IL-17A High Sensitivity ELISA (eBioscience, San Diego, CA) according to the manufacturer's instructions. Since it has been reported that tobacco smoke is related to Th17 generation in psoriasis patients, active smokers ($n = 1$ in HV, $n = 2$ in UC, naive, and $n = 1$ in CD, failure) were eliminated in this study.

Statistics

Statistical analysis was performed using SAS System for Windows (Release 8.2, SAS Institute, Tokyo, Japan) or the EXSUS statistical analysis system (8.0 ver, CAC EXICARE, Tokyo, Japan). Wilcoxon test, Student's *t* test and

Aspin–Welch's *t* test were performed as required. $p < 0.05$ were considered statistically significant. One-tailed Williams' test or Shirley–Williams' test were performed as required and $p < 0.025$ was considered statistically significant.

In vitro study by use of human PBMCs, statistical analysis was performed using GraphPad PRISM 5 for Windows. Mann–Whitney *U* test was performed as required, and $p < 0.05$ was considered statistically significant. To calculate IC_{50} values of TAK-828F, each data was further normalized to that of controls; data without the compound was defined as 100%, and that with the maximum concentration (1 μM) of the compound was defined as 0%. The IC_{50} values were determined by non-linear logistic regression.

Results

Effect of anti-TNF-α mAb and IL-12/23p40 mAb on naive T cell transfer colitis model

To validate the sensitivity of naive T cell transfer colitis model to representative anti-cytokine mAb, we investigated the protective efficacy of anti-TNF-α and IL-12/23p40 mAb in the colitis model. Anti-TNF-α mAb did not prevent onset of diarrhea and increase of colon weight (Fig. 1a, b). In contrast to anti-TNF-α mAb, anti-IL-12/23p40 mAb significantly inhibited onset of diarrhea and colon weight gain, respectively (Fig. 1c, d).

Pathogenesis of $CD4^+$ naive T cells prepared from $ROR\gamma^{-/-}$ mice on colitis

For the purpose to evaluate the impact of RORγt in the pathogenesis of naive T cell transfer colitis model, naive

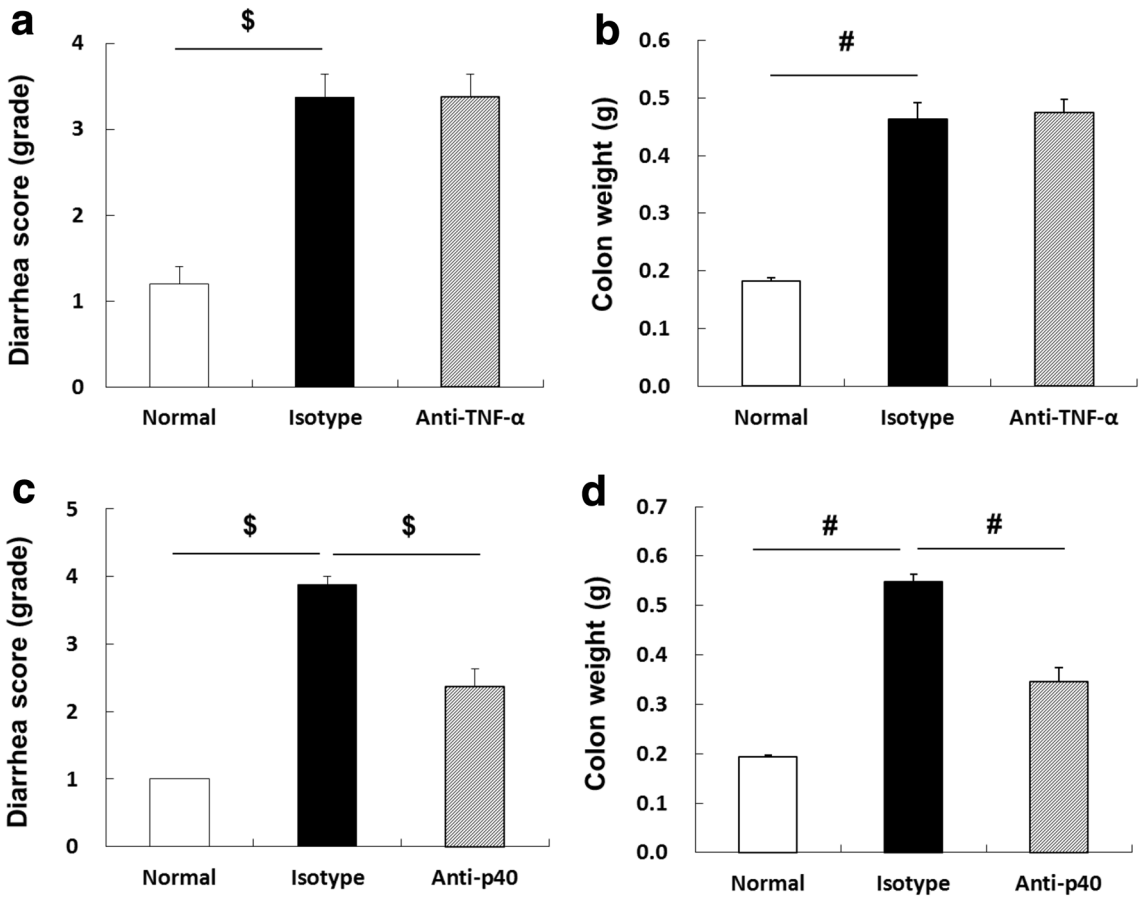

Fig. 1 Effect of anti-TNF-α mAb and anti-IL-12/23p40 mAb on naive T cell transfer colitis model. Anti-TNF-α mAb (**a**, **b**), anti-IL-12/23p40 (**c**, **d**) mAb or isotype control at dose of 0.1 mg/mouse were intraperitoneally administered to the SCID mice on day 0, 5, 9, 13 and 17 after naive T cell transfer. Diarrhea score (**a**, **c**) and colon weight (**b**, **d**) were analyzed under blind fashion. Data were represented as the mean ± SE. $^{\#}p < 0.05$ (Student's *t* test) and $^{\$}p < 0.05$ (Wilcoxon test) vs. normal group or control group

T cells, isolated from splenocyte of RORγ$^{-/-}$ or WT mice, were transferred to SCID mice and disease severity was observed. In the recipient mice transferred with naive T cells from RORγ$^{-/-}$ mice, disease severity was significantly decreased compared to the mice transferred with that of WT mice (Fig. 2a, b). Noteworthy, specific reduction of Th17 and Th1/17 cell population, but not Th1, were observed in the MLN of the mice transferred with naive T cells from RORγ$^{-/-}$ mouse (Fig. 2c–f), indicating that RORγ has essential role to regulate not only Th17 but also Th1/17 cell differentiation and contribute to the pathogenesis of colitis.

Effect of TAK-828F on naive T cell transfer colitis model

Protective efficacy of TAK-828F in the colitis model was investigated. TAK-828F was orally administered to the mice at the doses of 1 and 3 mg/kg twice a day. TAK-828F significantly inhibited onset of diarrhea and colon weight gain in a dose-dependent manner (Fig. 3a, b). In the histopathological study, TAK-828F at 1 and 3 mg/kg significantly reduced the total histopathological score of the colon (Fig. 3c). The mean total histopathological score for the vehicle-treated control group was 11.0 ± 2.6 and for 1 and 3 mg/kg of TAK-828F treatment groups were 7.7 ± 3.0 and 6.1 ± 1.6, respectively. TAK-828F reduced the severity and/or incident of infiltration of mononuclear and inflammatory cells, mucosal regeneration/hyperplasia and crypt abscesses/debris in the lumen (Fig. 3d–g).

Comparison of efficacy between TAK-828F and anti-IL-17A mAb in naive T cell transfer colitis model

Anti-IL-17A mAb failed in a clinical trial of CD [25]. Treatment of human and mouse immune cells by TAK-828F resulted in the inhibition of Th17 cell generation and Th17-related multiple cytokines production [23], indicating the difference of pharmacological effect between blocking IL-17A and RORγt. To elucidate the difference of pharmacological profile between blocking of IL-17A and RORγt in vivo, the efficacy of TAK-828F in the colitis model was directly compared to that of anti-IL-17A mAb. As shown in Fig. 4a, b, TAK-828F again ameliorated disease progression in the model. In the mice, TAK-828F significantly reduced Th17 and Th1/17 cell population in the MLN, but did not affect that of Th1 in the model (Fig. 5), as shown in the mice transferred naive T cells from RORγ$^{-/-}$ mice. To evaluate the effect of TAK-828F on effector function of T cells in the colon, we measured

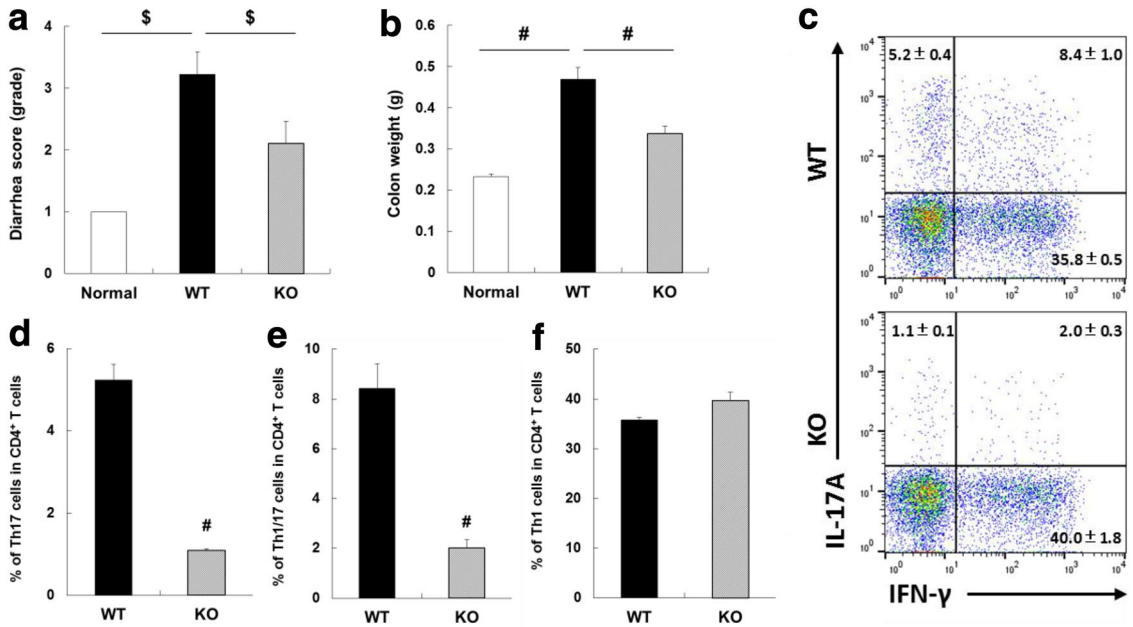

Fig. 2 Role of RORγ in the pathogenesis of the colitis model. Naive T cells, isolated from splenocyte of RORγ$^{-/-}$ (KO) and WT mice, were transferred to SCID mice and disease severity was evaluated on day 21 after naive T cell transfer. **a** Diarrhea score and **b** colon weight were analyzed in a blind fashion on day 21. Data were represented as the mean ± SE of 6 (normal) or 9 (transferred mice) animals. The frequency of Th17, Th1/17 and Th1 cells in MLNs was determined by flow cytometry. **c** Representative dot plot analysis of Th1, Th1/17 and Th17 cells in CD4$^+$ T cells. The frequency of **d** Th17 cells, **e** Th1/17 cells and **f** Th1 cells gated on CD4$^+$ T cells. Data were represented as the mean ± SE of three samples (MLNs from 3 animals were gathered into one sample). $^{\$}p < 0.05$ (Wilcoxon test) and $^{\#}p < 0.05$ (Student's *t* test) vs. wild type group

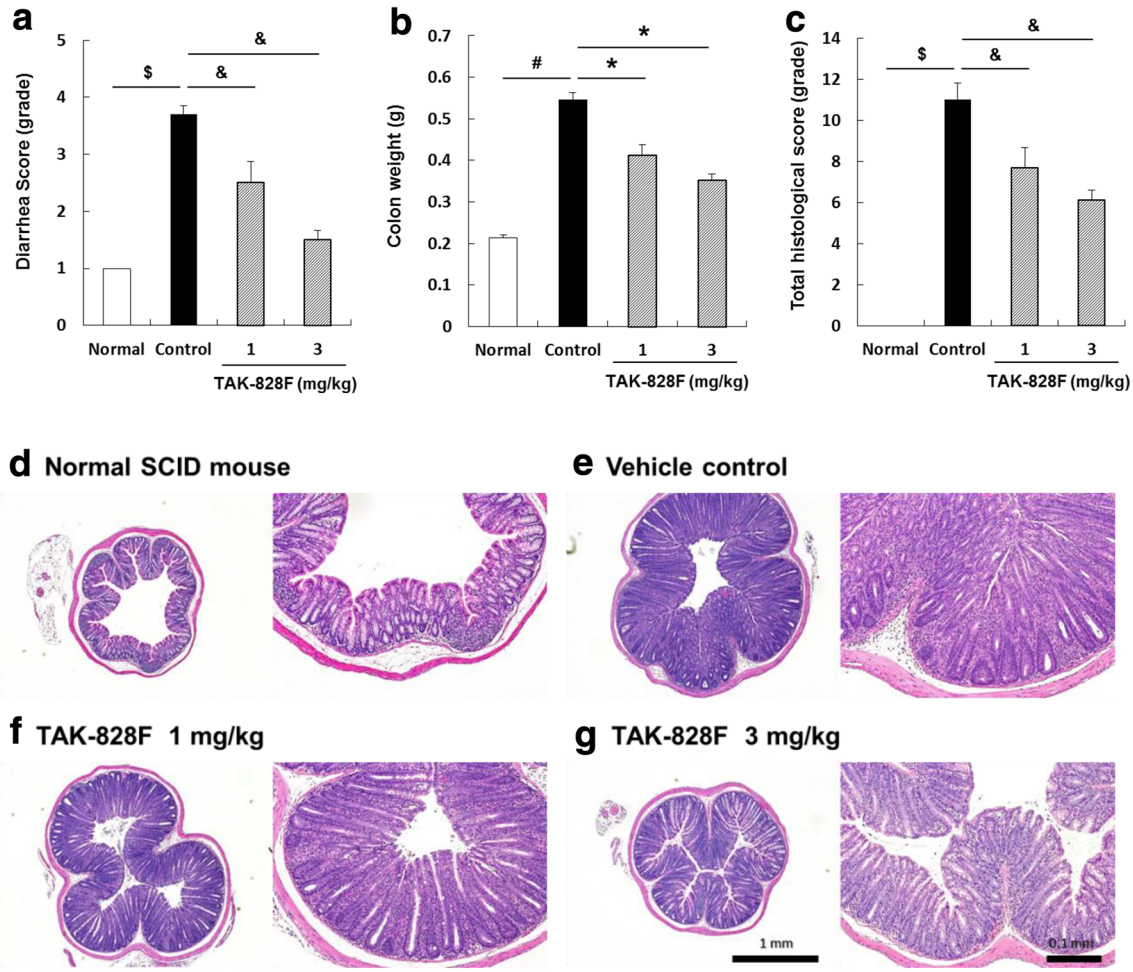

Fig. 3 Effect of TAK-828F on naive T cell transfer colitis model. TAK-828F (1 and 3 mg/kg) or vehicle (0.5% methylcellulose) was orally administered to the SCID mice twice a day for 21 days. **a** Diarrhea score, **b** colon weight, and **c** total histopathological score were analyzed. Histopathological score of the colon was determined by staining with H&E as **d** normal SCID mice, **e** vehicle control group, **f** TAK-828F at 1 mg/kg-treated group and **g** TAK-828F at 3 mg/kg-treated group. Data were represented as the mean ± SE of 5 (normal) or 10 (transferred mice) animals. $^{\$}p < 0.05$ (Wilcoxon test) and $^{+}p < 0.05$ (Aspin–Welch's *t* test) vs normal group. $^{\&}p < 0.025$ (one-tailed Shirley–Williams' test) and $^{*}p < 0.025$ (one-tailed Williams' test) vs vehicle control group

colonic gene expression of IL-17A, IL-17F and IL-22 that are characteristic of the Th17 signature, and IFN-γ that of the Th1 signature. In the colonic gene expression analysis, TAK-828F treatment resulted in the significant reduction of IL-17A, IL-17F and IL-22 expression (Fig. 6a–c). Colonic gene expression of IFN-γ was partially reduced by treatment with TAK-828F. Interestingly, TAK-828F treatment at a dose of 3 mg/kg significantly increased the gene expression of IL-10, an anti-inflammatory cytokine, in the colon. In contrast to TAK-828F, no significant efficacy was observed in the mice treated with anti-IL-17A mAb to neutralize IL-17A (Fig. 4). Anti-IL-17A mAb affected neither T cell frequency in the MLN nor colonic gene expression in the mice (Figs. 5 and 6).

Effect of TAK-828F on the immune cell population in normal mice

Immunomodulatory agents usually cause immunosuppression and sometimes induce the reduction of infection immunity in the patients [29]. In order to evaluate whether blocking RORγt activity causes systemic immunosuppression or not, we investigated the effect of TAK-828F on the immune cell population in normal mice. TAK-828F was administered to the normal mice at effective (3 mg/kg, b.i.d.) and excess (30 mg/kg, b.i.d.) doses for 4 weeks, lymphocytes prepared from the spleen and the mucosa of small intestine were measured by flow cytometry. Small intestine was used in the assay as described in "Materials and methods", since innate lymphoid 3 (ILC3) cells were mainly localized in

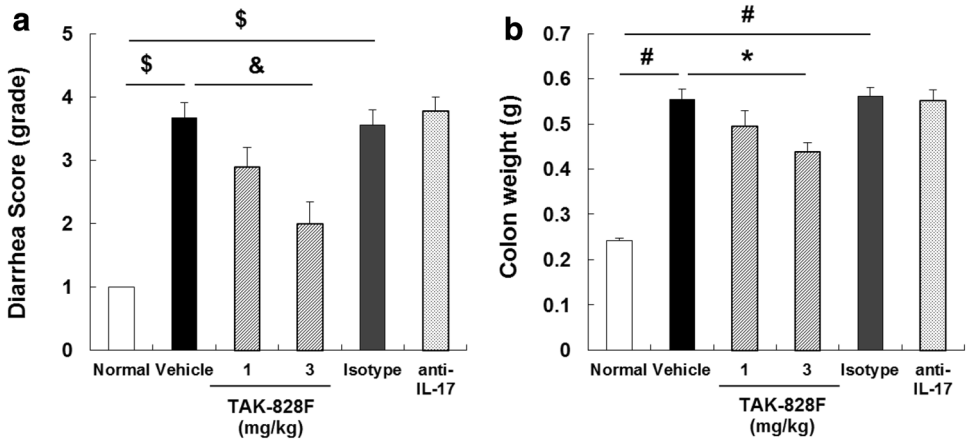

Fig. 4 Effect of TAK-828F and anti-IL-17A mAb on naive T cell transfer colitis model. TAK-828F (1 and 3 mg/kg) or vehicle (0.5% methylcellulose) was orally administered to the SCID mice twice a day for 21 days. Anti-IL-17A mAb or IgG isotype control Ab (20 μg/mouse) was intraperitoneally administered to mice at day 0, 7 and 14. **a** Diarrhea score and **b** colon weight were analyzed in a blind fashion on day 21. Data were represented as the mean ± SE of 5 (normal) or 9 (transferred mice). $^{\$}p < 0.05$ (Wilcoxon test) and $^{\#}p < 0.05$ (Student's *t* test) vs normal group. $^{\&}p < 0.025$ (one-tailed Shirley–Williams' test) and $^{*}p < 0.025$ (one-tailed Williams' test) vs vehicle control group

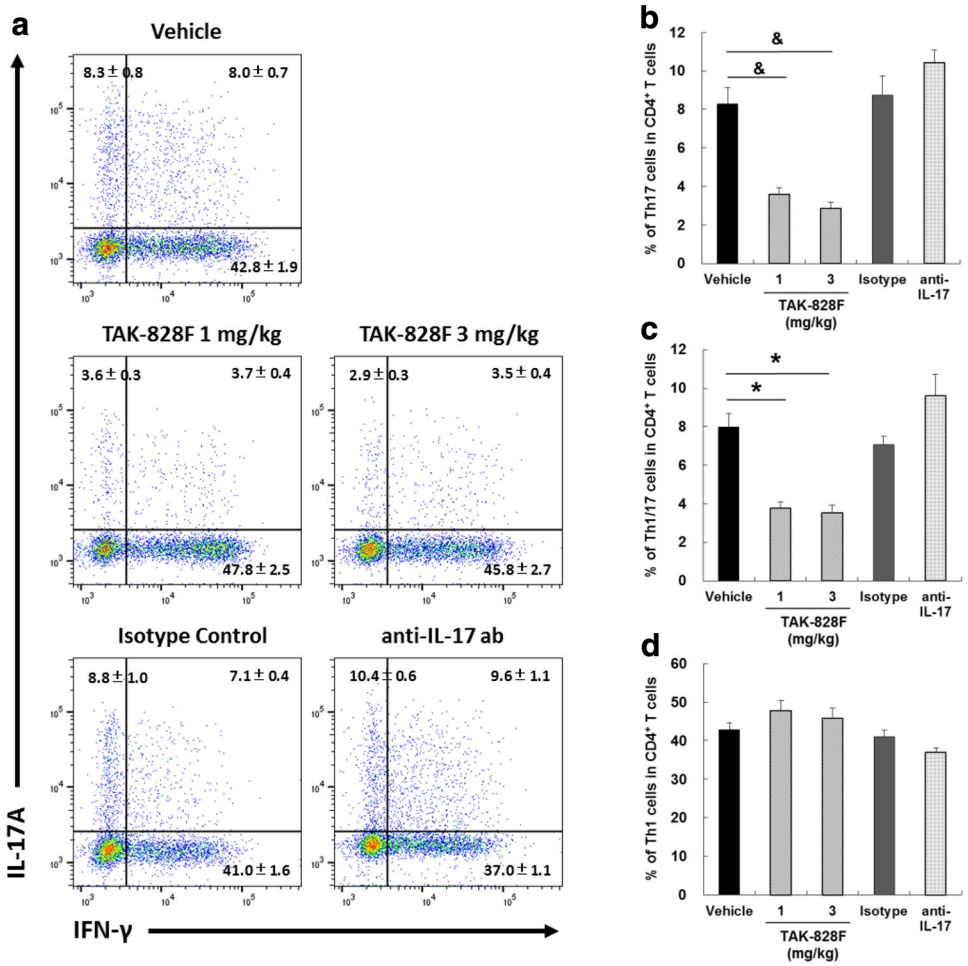

Fig. 5 Effect of TAK-828F and anti-IL-17A mAb on T cell population of naive T cell transfer colitis model. TAK-828F (1 and 3 mg/kg) or vehicle (0.5% methylcellulose) was orally administered to the SCID mice twice a day for 21 days. Anti-IL-17A mAb or IgG isotype control Ab (20 μg/mouse) was intraperitoneally administered to mice at day 0, 7 and 14. The frequency of Th17, Th1/17 and Th1 cells in MLNs were determined by intracellular staining. **a** Representative dot plot analysis of Th1, Th1/17 and Th17 cells in $CD4^+$ T cells. The frequency of **b** Th17 cells, **c** Th1/17 cells and **d** Th1 cells gated on $CD4^+$ T cells. Data were represented as the mean ± SE of 9. $^{\&}p < 0.025$ (one-tailed Shirley–Williams' test) and $^{*}p < 0.025$ (one-tailed Williams' test) vs vehicle control group

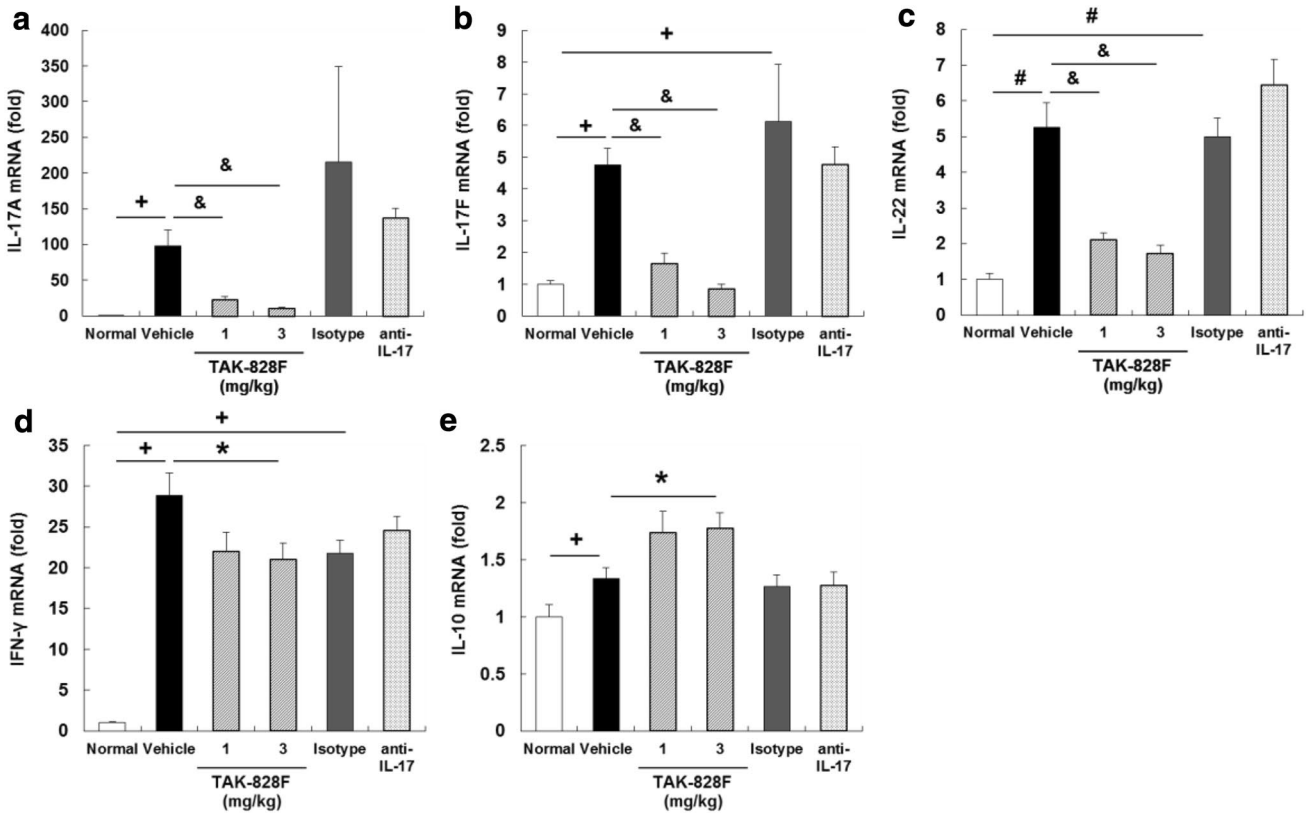

Fig. 6 Effect of TAK-828F and anti-IL-17A mAb on colonic gene expressions in naive T cell transferred mice. Total RNA was isolated from colonic tissue on day 21 and relative mRNA expression of **a** IL-17A, **b** IL-17F, **c** IL-22, **d** IFN-γ and **e** IL-10 in colon was measured by real-time RT-PCR, normalized with the expression of β-Actin. Data were represented as the mean ± SE of 5 (normal) or 9 (transferred mice). $^{\#}p < 0.05$ (Student's *t* test) and $^{+}p < 0.05$ (Aspin–Welch's *t* test) vs normal group. $^{\&}p < 0.025$ (one-tailed Shirley–Williams' test) and $^{*}p < 0.025$ (one-tailed Williams' test) vs vehicle control group

the small intestine of the mice. Spleen cells were used as peripheral cells in the assay as described in the methods. TAK-828F, at any dose, did not decrease the peripheral lymphocytes cell population of male mice (Fig. 7). In female mice, TAK-828F showed no dose-dependent reduction of peripheral lymphocyte cell population, though partial reduction of total $CD4^+$, $CD8^+$, naive $CD4^+$ and memory $CD4^+$ cells was observed at an effective dose. In the small intestine, TAK-828F dose-dependently reduced RORγt-positive Th17, ILC3 and its subtype (NCR^+ ILC3 and NCR^- ILC3) cells. In addition to the reduction in these cell types, $CD4^+$ and $CD8^+$ cell populations in female mice were decreased by the treatment of excess dose of TAK-828F (30 mg/kg, b.i.d.), but counts of other cell types were not affected. Thus, effects of TAK-828F on immune cell fractions in periphery and small intestine were limited.

Next, the immune cell population in the mice of $ROR\gamma^{-/-}$ mice was compared to that of WT mice (Fig. 8). The populations of $CD4^+$, $CD8^+$, NK and naive $CD4^+$ cell were significantly decreased in the periphery of $ROR\gamma^{-/-}$ mice compared with those in WT mice. In the small intestine, Th17, ILC3 and its subtypes (NCR^+ and NCR^- ILC3) cell population significantly decreased in the $ROR\gamma^{-/-}$ mice. In addition to these cell species, a significant reduction of $CD4^+$, B, naive $CD4^+$, memory $CD4^+$ and activated $CD4^+$ cell population was observed in $ROR\gamma^{-/-}$ mice. Therefore, $ROR\gamma^{-/-}$ mice have severe perturbations of immune cell fractions in both periphery and gut.

Effects of TAK-828F in PBMCs from IBD-patients

Finally, we have evaluated the inhibitory effects of TAK-828F in human PBMCs, as well as whether RORγt-related pathways contribute to the sensitivity of anti-TNF failure in IBD, by using blood samples of IBD patients. For this purpose, a total of 13 UC patients, 12 CD patients, and 5 healthy volunteers (HV) were recruited to perform this study, and they were further divided into anti-TNF naïve or failure subgroups; baseline characteristics of these patients were summarized in Table 1.

First, we evaluated both plasma concentration of IL-17a and CD3/CD28-induced gene expression of IL-17a in the PBMCs. IL-17a was used as an activation marker of RORγt. Although the IL-17a mRNA induction in PBMCs

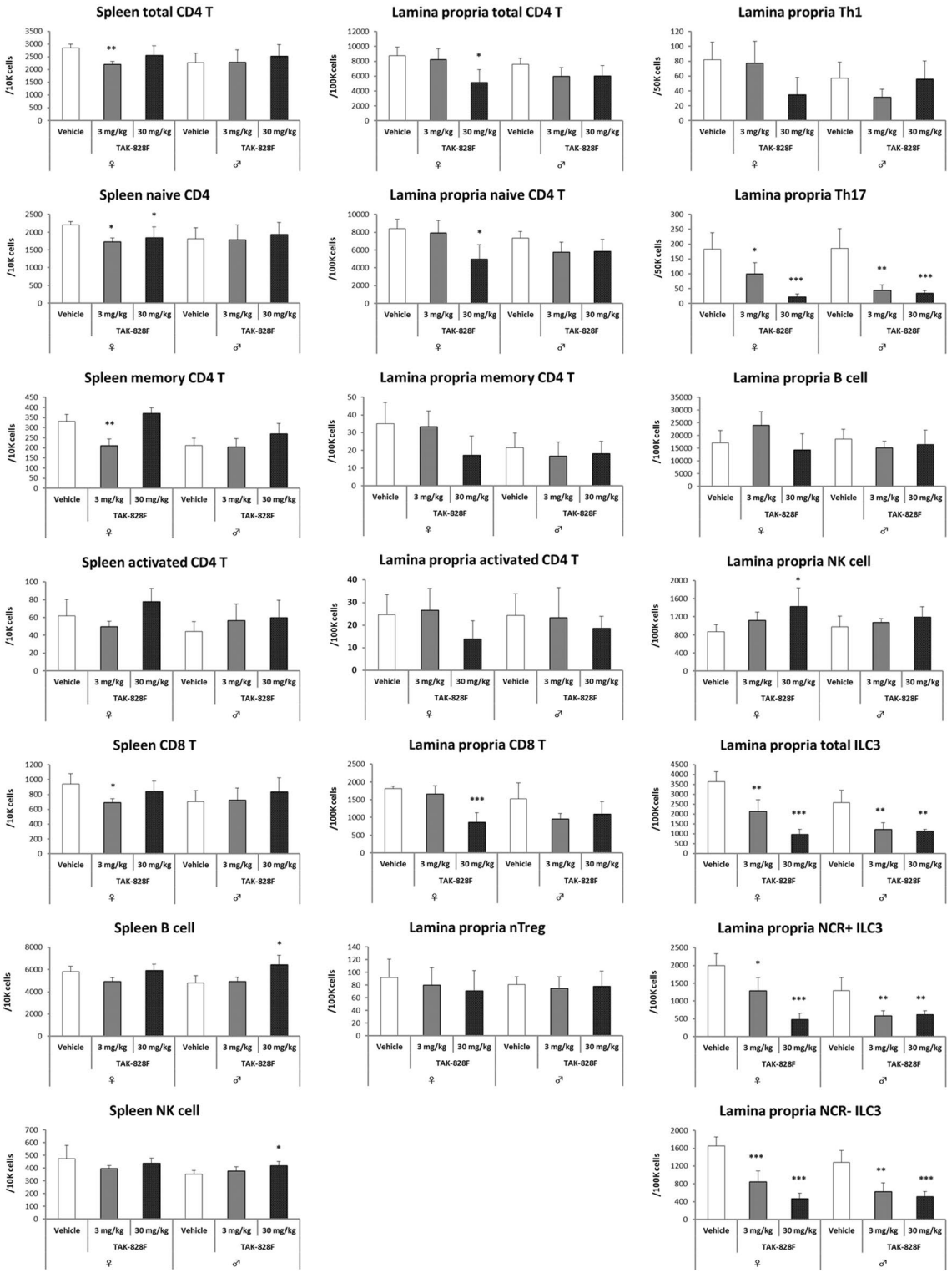

Fig. 7 Effect of TAK-828F on immune cell population of normal mice. Normal mice were administered with TAK-828F (3 or 30 mg/kg, b.i.d.) for 28 days. Then the population of each type immune cells was analyzed by flow cytometry. Absolute cell number per 10000 cells in spleen, absolute cell number per 100,000 cells in small intestine (except for Th1 and Th17) and absolute number per 50,000 cells in small intestine (Th1 and Th17) were indicated. Data was represented as mean ± SE of 4. $^{*}p < 0.05$ (William's *t* test) vs. vehicle control group

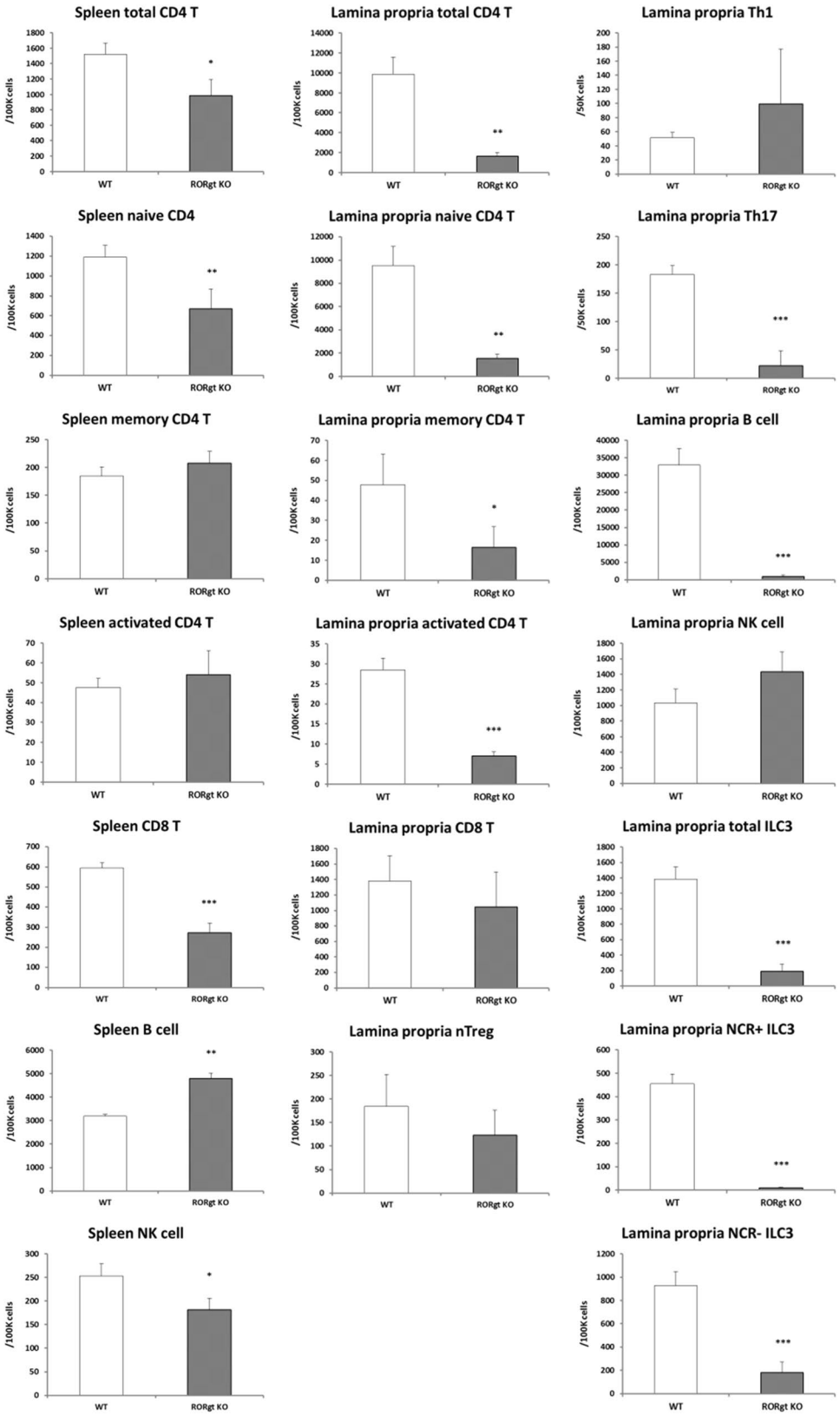

Fig. 8 Comparison of immune cell population between normal and RORγ$^{-/-}$ mice. The population of each type immune cells was analyzed by flow cytometry using wild type and RORγ$^{-/-}$ mice. Absolute cell number per 10,000 cells in spleen, absolute cell number per 100,000 cells in small intestine (except for Th1 and Th17) and absolute number per 50,000 cells in small intestine (Th1 and Th17) were indicated. Data was represented as mean ± SE [$n = 4$ (wild type mice), $n = 3$ (RORγ$^{-/-}$ mice)]. $^{\#}p < 0.05$ (Student's t test) vs. wild type group

Table 1 Characteristics of HV and IBD patients included in this study

	HV	UC	CD
N	5	13	12
Age (mean ± SD) (years)	34.2 ± 3.63	38.69 ± 16.92	27.58 ± 8.44
Gender (male/female)	4/1	5/8	6/6
Disease duration (mean ± SD) (years)	n.a.	8.85 ± 7.72	7.67 ± 7.50
Anti-TNF treatment			
Naive	n.a.	11	5
Failure	n.a.	5[a]	9[b]
Smoking status, *n* (%)			
Non smoker	3 (60.0)	4 (30.1)	9 (75.0)
Smoker (past)	1 (20.0)	2 (15.4)	1 (8.3)
Smoker (active)	1 (20.0)	2 (15.4)	1 (8.3)
Unknown	0 (0.0)	5 (38.5)	1 (8.3)

[a]Three patients were also included in naive-group

[b]Two patients were also included in naive-group

(Fig. 9c, d) was not significantly different between HV and IBD patients, the concentration of IL-17a in plasma (Fig. 9a, b) was significantly higher in UC (both anti-TNF naive and failure) and CD (anti-TNF failure) than that in HV. Interestingly, in CD patients, induction of IL-17a mRNA in PBMC was increased in anti-TNF failure compared to naive patients (Fig. 9d). The same tendency could be seen in plasma IL-17a concentration, although it was not significantly different ($p = 0.10$, Fig. 9b).

Next, we evaluated the inhibitory effects of TAK-828F against the CD3/CD28-induced IL-17a gene expression in PBMCs. As shown in Fig. 10, TAK-828F inhibited the gene expression of IL-17a in a concentration-dependent manner in the PBMCs from IBD patients as in those from HV. An IC_{50} value of TAK-828F against PBMCs from HV was 26.4 nmol/L, and anti-TNF naive, or anti-TNF failure of IBD patients were from 21.4 to 34.4 nmol/L (Fig. 10a, b). No significant inhibition in the gene expression of IFN-γ in the PBMCs by TAK-828F was observed even at 1000 nmol/L (data not shown). These results indicated that TAK-828F strongly inhibits RORγt-dependent IL-17 gene expression in PBMCs both in anti-TNF naive and failure patients of IBD.

Discussion

Before conducting in vivo efficacy study of TAK-828F in the naive T cell transfer colitis model, we have investigated the sensitivity of the model to typical monoclonal antibody, such as ant-TNF-α mAb and anti-IL-12/23p40 mAb. The colitis model showed less sensitivity to anti-TNF-α mAb (Fig. 1a, b) despite of the sufficient plasma concentration of the mAb in mice as described in "Materials and methods". In contrast, anti-IL12/23p40 mAb showed significant efficacy in the model (Fig. 1c, d). Clinically, ustekinumab, an anti-IL-12/23p40 mAb, shows efficacy in the anti-TNF-α

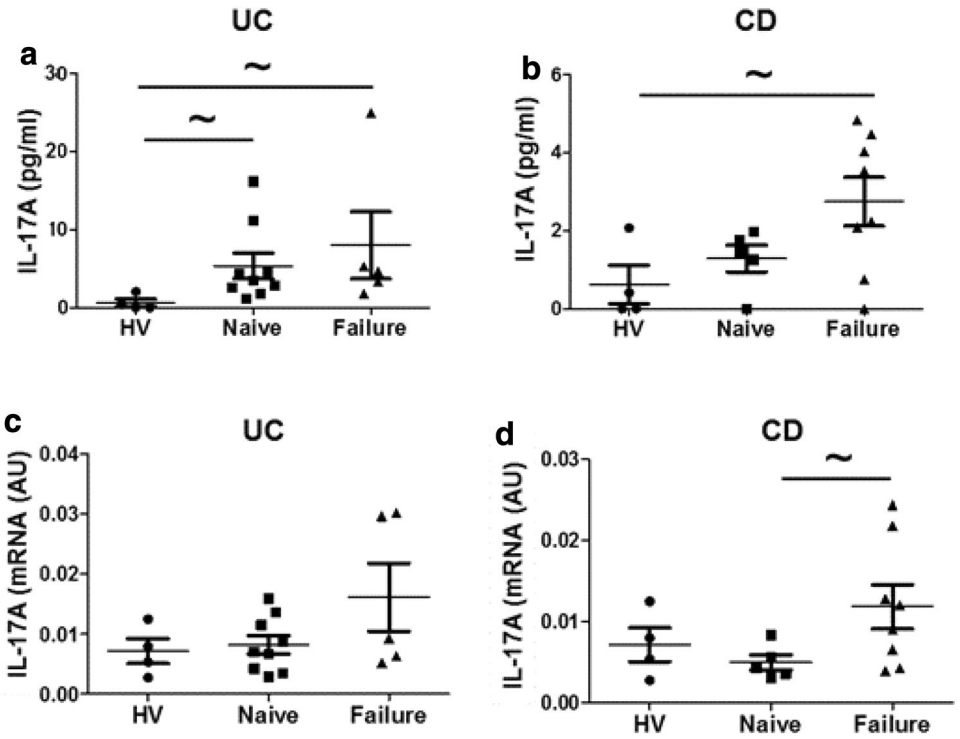

Fig. 9 IL-17A in IBD patient-oriented blood samples. Plasma IL-17A concentration (**a**, **b**) and anti-CD3/CD28-induced IL-17A mRNA expression in PBMCs (**c**, **d**) were evaluated in HV, UC (**a**, **c**) and CD (**b**, **d**) patients. As explained in "Materials and methods", active smokers ($n = 1$ in HV, $n = 2$ in UC, and $n = 1$ in CD) were eliminated in this study. Data were represented as the mean ± SE. ~$p < 0.05$, Mann–Whitney *U* test

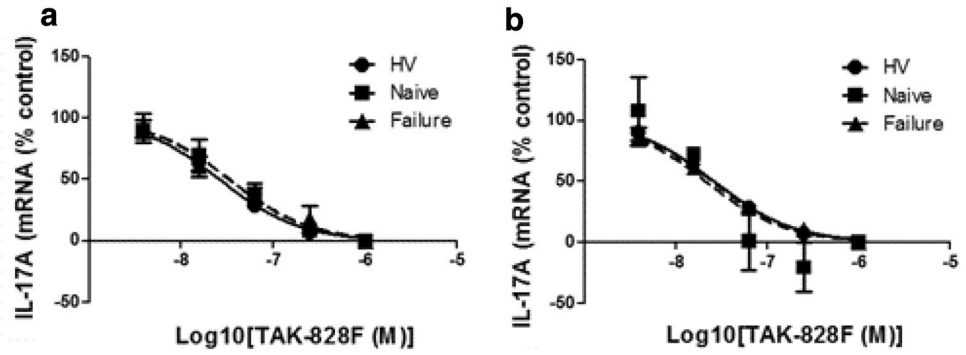

Fig. 10 Inhibitory effects of TAK-828F against the induction of IL-17A mRNA in PBMCs of UC and CD patients. Total RNA was isolated from PBMCs 20 h after stimulation with CD3/CD28 antibodies, and relative IL-17A mRNA expression was measured by real-time PCR. Each data was normalized to that of controls; data without the compound was defined as 100%, and that with the maximum concentration (1 μM) of the compound was defined as 0%. The IC_{50} values were determined by non-linear logistic regression in UC (**a**) and CD (**b**): 26.4 nmol/L (HV, $n=5$), 34.4 nmol/L (anti-TNF naive UC, $n=11$), 33.5 nmol/L (anti-TNF failure UC, $n=5$), 21.4 nmol/L (anti-TNF naive CD, $n=5$), and 25.0 nmol/L (anti-TNF failure CD, $n=9$)

mAb-refractory CD patients [30]. From these facts, we consider that naive T cell transfer colitis model might be useful to evaluate candidate compounds for indication of severe CD in patients who are refractory to anti-TNF-α mAb therapy.

To evaluate the involvement of RORγt in the pathogenesis of naive T cells transfer colitis model, we compared the severity of colitis induced by naive T cells derived from RORγ$^{-/-}$ mice and WT mice. Disease severity induced by naive T cells from RORγ$^{-/-}$ mice was less than that induced by naive T cells from WT mice (Fig. 2). In the mice transferred with naive T cells from RORγ$^{-/-}$ mouse, Th17 and Th1/17 cell population in the MLN was significantly reduced in comparison with naive T cells from WT mice induced colitis, but Th1 cell population was not affected. These findings corresponded to the study of the colitis model induced by transfer of naive T cells isolated from IL-23R$^{-/-}$ mice [31]. In addition, it is reported that IL-23 plays a critical role in Th17 cell differentiation [14, 15]. These results indicated that RORγt has a high contribution to the pathogenesis of the colitis model by accelerating Th17 and Th1/17 cell differentiation and pharmacological role of RORγt in the colitis is well overlapping to that of the IL-23/Th17 pathway.

In the colitis model, oral treatment of TAK-828F at doses of 1 and 3 mg/kg significantly prevented onset of colitis (Figs. 3 and 4). In the histopathological analysis of the colon, TAK-828F reduced intestinal inflammation and mucosal hyperplasia in the lumen. These results suggest that TAK-828F exhibits a beneficial effect on prevention of mucosal damage caused by intestinal inflammation. Consistent with our findings, Withers et al. [32] reported that GSK805, a selective RORγt inverse agonist, showed efficacy in a similar adoptive T cell transfer model (CBir1 TCR transgenic T cells into RAG1$^{-/-}$ mice). However, the sensitivity of the colitis against anti-TNF-α and IL-12/23p40 mAb has not been investigated in their model. Thus, it is the first time, to our knowledge, to demonstrate that a RORγt inverse agonist was efficacious in the anti-TNF-α mAb non-responding colitis model.

Th17 and Th1/17 cell populations, but not Th1, in the MLN were significantly decreased by the treatment of TAK-828F in the model (Fig. 5), which is the same observation in the mice that transferred naive T cells isolated from RORγ$^{-/-}$ mice (Fig. 2). In vitro assay by using primary human memory T cells, TAK-828F also specifically inhibited Th17 and Th1/17 cell differentiation but did not affect Th1 differentiation [23]. In addition to the reduction of Th17 cells in MLN by treatment with TAK-828F in the colitis model, TAK-828F dose-dependently inhibited IL-23-related gene expression, such as IL-17A, IL-17F and IL-22 in the colon of mice (Fig. 6). These results suggest that TAK-828F can block IL-23/Th17 axis in vivo by selectively inhibiting RORγt.

As shown in Fig. 6, remarkable reduction of Th17 signature gene expression, but partial reduction of Th-1 signature gene expression, in the colon was observed by treatment with TAK-828F, suggesting that Th17 cells strongly exert their effector function in the colon of mice and TAK-828F specifically inhibits the function. Although we did not measure T cell population in the colon, the colonic T cell population might be similar to that observed in MLN, because strong reduction of Th-17-related gene expression, but not Th1-related gene expression was observed in the colon by treatment with TAK-828F. The partial reduction of colonic gene expression of IFN-γ by treatment with TAK-828F might be due to the significant reduction of Th1/17 cells which produce IFN-γ.

In contrast to TAK-828F, anti-IL-17A mAb was ineffective in the model and does not affect the cell population of Th17 and Th1/17 in the MLN and the colonic gene expression that are characteristic of the Th-17 signature. Moreover,

Maxwell et al. [33] reported that IL-17 inhibition exacerbated colitis of multidrug resistance-1a-ablated (Abcb1a$^{-/-}$) mouse model whereas IL-23 inhibition attenuated disease severity of that model by decreasing frequency of Th17 and Th1/17 cells. Therefore, we can conclude that pharmacological profile of TAK-828F is clearly different from IL-17 inhibitor.

Although IL-17 and IL-17 receptor (IL-17R) mAb, secukinumab and brodalumab, are clinically efficacious in psoriasis [34], these mAbs against CD show no beneficial effect or exacerbation of disease [25]. IL-17A is reported to have a key role to maintain intestinal tight junction via the IL-17 receptor adaptive protein Act-1; therefore, complete blockage of IL-17 signal by anti-IL-17 and anti-IL-17R mAb is considered to one of the possible reason of exacerbation in CD [35]. In the intestine, IL-17 is produced by not only Th17 but also ILC3 cells, and gene expression of IL-17 in ILC3 is regulated by other transcription factor such as GATA-3 and aryl hydrocarbon receptor [36] [37]. Actually, the colonic gene expressions of IL-17A and IL-17F were not completely inhibited by treatment with TAK-828F in the colitis model (Fig. 6a, b) and TAK-828F protected mucosal damage in the histopathological analysis (Fig. 3c). From these results, we could expect that blockage of RORγt in CD patients would not induce the exacerbation of symptoms observed in anti-IL-17 mAb therapy.

TAK-828F showed limited effect on immune cell population in normal mice compared with RORγ$^{-/-}$ mice (Figs. 7, 8). Recently, bi-allelic RORγ loss of function mutations (RORγ$^{-/-}$) in human has been reported and suggests that mucocutaneous immunity to Candida and systemic immunity to Mycobacterium require RORγ or RORγt, or both [38]. RORγ$^{-/-}$ patients displayed a severe reduction of peripheral MAIT, type1 NKT, ILC3 and Th17 cells along with mild T cell lymphopenia and these immunological features were similar to that observed in RORγ$^{-/-}$ mice [38]. In our experiments, a significant reduction of multiple lymphocytes both in peripheral and intestine was observed in RORγ$^{-/-}$ mice, though we did not measure peripheral MAIT, type 1 NK cell population. In contrast, administration TAK-828F at 3 mg/kg, an effective dose in the colitis mode, to normal mice for 4 weeks showed little effect on other peripheral lymphocytes and TAK-828F specifically reduced cell population of only Th17 and ILC3 cells. These results indicate that impact to the immune cell population by transient blockade of RORγt after birth is lower than that in the lack of RORγt function at the development stage. In regard to ILC3 in human, effect of RORγt inhibition on the human ILC3 cells might be limited, since GSK805 only reduced Th17 cells, but did not alter ILC3 frequencies in the ex vivo culture of colonic resection tissues of CD patients [32]. Although the numbers of some immune cells (e.g. B cells and NK cells) other than Th17 and ILC3 tended to differ between males and females in the TAK-828 administration study, we speculate that it was due to the small number of mice in each group ($n = 4$).

Plasma level of IL-17a from anti-TNF failure in UC and CD patients was higher than that from HV (Fig. 9a, b). In addition, increase in the gene expression of IL-17a in PBMCs was also detected in anti-TNF failure UC and CD patients compared with HV, though it was not significant in UC (Fig. 9c, d). Interestingly, gene expression of IFN-γ in PBMCs from UC and CD patients were decreased compared with that of HV (data not shown). These results suggest that up-regulation of the RORγt-related pathway in PBMCs is involved in the pathogenicity of IBD, especially in the patients of anti-TNF failure. The increase in the plasma level of IL-17A in the patients of TNF failure also supports the hypothesis that RORγt pathway may contribute to the resistance to anti-TNF mAb. In order to clarify this hypothesis, additional studies using PBMCs from more number of patients and HV is necessary.

IC_{50} values of TAK-828F against PBMCs from anti-TNF naive and anti-TNF failure of UC and CD patients were around 30 nmol/L and they were almost similar to that of HV (Fig. 10a, b). These data indicate that TAK-828F can comparably suppress RORγt-related pathways not only in anti-TNF naive patients, but also in anti-TNF failure patients. These evidences suggest that blockade of RORγt is a strategic way to manage the symptoms of UC and CD patients including anti-TNF non responder.

To summarize, TAK-828F was efficacious in the naive T cell transfer colitis model that is non-responding to anti-TNF-α mAb and pharmacological profile of TAK-828F in the model was different from that of anti-IL-17A mAb. In addition, TAK-828F inhibited ex vivo Th-17-related gene expression in PBMC from anti-TNF-α mAb naive and failure patients of IBD. RORγt blockade by oral small molecular compound would provide the unique approach for treating anti-TNF-α mAb refractory patients of IBD by blocking IL-23/Th17 pathway without severely affecting systemic immunity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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