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Tyrosine Kinase 2 Signalling Drives Pathogenic T cells in Colitis

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Abstract

Background and Aims: Tyrosine kinase 2 [TYK2] is required for the signalling of key cytokines in the pathogenesis of inflammatory bowel disease [IBD]. We assessed the efficacy of a novel selective TYK2 inhibitor [TYK2i] in experimental colitis, using pharmacological and genetic tools.

Methods: At onset of T cell transfer colitis, RAG1$^{-/-}$ mice received vehicle or TYK2i daily by oral gavage. T cells lacking TYK2 kinase activity [TYK2^{KE}] were used to confirm selectivity of the inhibitor. To this end, RAG1$^{-/-}$ or RAG1$^{-/-}$TYK2^{KE} animals were transferred with either wild type [WT] or TYK2^{KE}-CD45RB^{high} colitogenic T cells. Loss of body weight, endoscopic disease, the disease activity index [DAI], and histopathology scores were recorded. Tissues were analysed *ex vivo* for lymphocyte populations by flow cytometry. The impact of TYK2 inhibition on human DC-T cell interactions were studied using autologous Revaxis specific T cell assays.

Results: TYK2i [70 mg/kg] prevented weight loss and limited endoscopic activity during T cell transfer colitis. TYK2i [70 mg/kg] decreased DAI. Whereas transfer of WT T cells into RAG$^{-/-}$TYK2^{KE} hosts induced colitis, TYK2^{KE} T cells transferred into RAG1$^{-/-}$TYK2^{KE} recipients failed to do so. *Ex vivo* analysis showed a decrease in colon tissue Th1 cells and an increase in Th17 cells upon transfer of TYK2^{KE}-CD45RB^{high} cells. In human antigen-triggered T cells, TYK2i displayed reduced Th1 differentiation, similar to murine Th1 cells.

Conclusions: Oral administration of TYK2i, as well as transfer of T cells lacking TYK2 activity, reduced human Th1 differentiation and ameliorated the course of murine T cell transfer colitis. We conclude that TYK2 is a promising drug target for the treatment of IBD.

Key Words: Tyrosine kinase 2 inhibitor; Janus kinase inhibitor; experimental colitis; IBD

1. Introduction

Inflammatory bowel disease [IBD] is characterised by chronic, relapsing inflammation of the intestine.[1,2] Although treatment options have expanded with the introduction of the anti-integrin vedolizumab and the biologic ustekinumab, unmet needs remain significant.[3,4] Janus kinase [JAK] inhibitors are emerging as novel therapy in IBD.[5–7] JAK1, JAK2, JAK3 and tyrosine kinase [TYK2] activate signal transducers and activators of transcription [STAT] family members. Upon activation by cytokines and growth factors, STAT complexes migrate to the nucleus, where they activate transcription.[8] Tofacitinib, a JAK inhibitor approved for the treatment of ulcerative colitis [UC], targets multiple JAK family members including JAK1, JAK3, and to a lesser extent JAK2 and tyrosine kinase 2 [TYK2].[5] Although JAK2 inhibition may lead to unwanted suppression of haematopoiesis in some indications, JAK1/2 inhibitors [ruxolitinib and baricitinib] are currently used for the treatment of myelofibrosis, rheumatoid arthritis [RA], atopic dermatitis, and systemic lupus erythematosus [SLE].[9,10] The results of treatment with the JAK1 selective inhibitors filgotinib and upadacitinib in Crohn's disease are promising, leading to further investigation of selective JAK1, JAK3, and TYK2 inhibition in the treatment of IBD. [6,11–13]

TYK2-dependent signalling is triggered by interferon [IFN] α and β, and by various cytokines involved in the pathogenesis of IBD, including interleukin [IL]6, IL10, IL12, IL13, IL22, and IL23 [reviewed in Srobl *et al.*[14]]. In TYK2-deficient macrophages, a reduced IFNα/β response was observed as well as a decrease in production of nitric oxide in response to toll-like receptors.[15] Others have established that IL12- and IL23-induced IFNγ production was diminished in TYK2-deficient dendritic cells, suggesting that TYK2 signalling is essential in the production of such Th1-promoting cytokines.[16] Furthermore, TYK2 is required for the expansion and maturation of Th17 cells through activation of IL23R-STAT3 signalling,[17] as well as the production of IL17, as demonstrated in TYK2-deficient animals.[18] These results support that TYK2 plays a non-redundant role in innate and adaptive immune responses relevant to IBD pathogenesis.

We hypothesised that targeting TYK2 kinase activity might have therapeutic potential for the treatment of IBD. Due to the high degree of sequence homology between the JAK family kinases, the development of highly selective TYK2 inhibitors has proven challenging. Three TYK2-selective inhibitors were described earlier: CMP6, Cmpd1, and NDI-031301.[19–21] The crystal structure of JAKs has been investigated in relation to tofacitinib and the JAK inhibitor pyridine-containing tetracycle CMP6.[22] Similarity in the crystal structures of the adenosine triphosphate [ATP]-binding pockets of all JAK isozymes hampers the generation of small molecule inhibitors exhibiting a high degree of selectivity, preventing development of inhibitors of the TYK2 molecule selectively.[20,23] Using a structure-based drug design programme, subsequent TYK2 inhibitors were generated [including Cmpd1], demonstrating a 100-fold biochemical selectivity over JAK2.[19] This, and other data obtained on the structure of TYK2, served as a platform for the development of the highly selective orally available TYK2 small molecule inhibitor, TYK2i, used in the current experiments.[24,25]

In this study, we aimed to investigate the role of TYK2 inhibition in the T cell transfer model of colitis, by making use of a novel orally available TYK2 inhibitor. Among many models for colitis, the T cell transfer model of colitis is a widely used model to dissect the initiation, induction, and regulation of immunopathology in chronic colitis mediated by T cells, and is established to share many critical features of human IBD[26]. We determined the cellular contribution of TYK2 signalling to the disease process by using animals carrying a point mutation in the ATP-binding pocket of the TYK2 kinase. We establish that TYK2i is a functional TYK2 inhibitor *in vivo* and that TYK2 signalling is a critical determinant of Th1 colitogenic T cells.

2. Materials and Methods

2.1. Reagents

The pharmacokinetics and specificity of the TYK2 inhibitor used was previously reported, designated TYK2i in this study and compound 37 in the previous study.[25]. Selectivity of this TYK2i over JAK1-3 was further described in Sohn *et al.* and Liang *et al.*[27,28] For *in vitro* experiments, TYK2i was dissolved in 100% dimethyl sulphoxide [DMSO; Sigma, Zwijndrecht, The Netherlands] at a concentration of 10 mM, and stored at -20C° until use. For 10 to 10 000 nM TYK2i concentrations, the stock was diluted with RPMI medium containing L-glutamine [L-Glut], fetal bovine serum [FBS; Sigma] and penicillin/streptomycin [Pen Strep; Thermofisher Scientific, Landsmeer, The Netherlands].

2.2. Animals

For T cell transfer experiments, female B6.129S7-Rag < tm1Mom>/J [RAG1$^{-/-}$] mice were purchased from Jackson Laboratories [Bar Harbor, ME, USA] and further bred in the animal facility of the Academic Medical Center [AMC], Amsterdam. As wild type donors, female C57BL/6 inbred mice were purchased from Charles River Laboratories [Maastricht, The Netherlands]. For *in vitro* and subsequent transfer experiments, two different TYK2-deficient mice were provided by the University of Veterinary Medicine, Vienna, Austria: mice carrying a point mutation in the TYK2 kinase domain [TYK2^{K923E}; termed henceforward TYK2^{KE}] resulting in abrogated kinase activity, as has been shown previously.[29,30] In addition, we were provided with mice carrying a targeted disruption of the TYK2 gene by placement of a neomycin resistance gene cassette [termed TYK2^{KO}].[15] For the second transfer experiment, TYK2^{KE} mice were back-crossed into RAG1$^{-/-}$ mice for three generations. Animals were housed in individually ventilated cages at the AMC's laboratory animal facilities. In all transfer experiments, acceptor and donor mice were age-matched [8–12 weeks] and gender-matched [female]. Animals were maintained on a 12/12 light/dark cycle under conditions of constant temperature [20°C ± 2°C] and humidity [55%] and were supplied with water and chow *ad libitum*.

2.3. T cell transfer colitis

We applied the T cell transfer model as described previously.[31] In short, wild type CD4^{+}CD45RB^{high} T cells were isolated from the spleen using magnetic depletion and flow cytometric cell sorting; 3 x 10^{5} cells were transferred into RAG1$^{-/-}$ mice by intraperitoneal injection. Control animals received no T cell transfer. Endoscopy was performed 3, 5, and 7 weeks after transfer, to assess the onset of disease and to monitor disease progression. In the first transfer experiment, cages were randomised [using an online randomiser: [www.randomizer.org]] for different treatments, although treatment was allocated per cage because of possible faecal-oral transfer of TYK2i. After positive endoscopy confirming onset of colitis, mice received oral gavage daily with 10 mg, 30 mg, or 70 mg/kg TYK2i, dissolved in 1% methylcellulose [Sigma]. These doses were selected based on previously generated pharmacokinetic data [internal GlaxoSmithKline-generated datasets]. In a subsequent experiment, RAG1$^{-/-}$TYK2^{KE} back-crossed mice were used as recipients of either WT or TYK2^{KE} T cells; these treatments were randomized within

cages. In a third experiment, three experimental interventions were performed: no transfer, transfer of WT T cells, or transfer of $TYK2^{KE}$ T cells into $RAG1^{-/-}$ recipients. This was randomised within cages. Weight was monitored three times per week. Upon sacrifice, the disease activity index [DAI] was determined by the level of diarrhoea [score 0–3], colonic inflammation/oedema [0–3], and the presence of blood in the stool [0–3], as previously described.[32] Histopathology and endoscopy scoring was performed by a blinded observer. All animal experiments were approved by and performed according to the guidelines of the Ethical Animal Research Committee of the University of Amsterdam, and were carried out in accordance with European Directive 2010/63/EEC and the GSK Policy on the Care, Welfare, and Treatment of Animals.

2.4. Histopathology

Histological scoring is the primary outcome of the T cell transfer model. Upon sacrifice, the colon was washed and cut longitudinally in half, and one part was processed for histology. Tissue was rolled into Swiss rolls and routinely embedded in paraffin; 4-µm sections were cut and stained by haematoxylin and eosin [HE] staining. Sections were then blinded, randomised, and scored for the following parameters: goblet cell loss [0–3], crypt loss [0–2], hyperplasia [0–3], and submucosal inflammation [0–3]. For each item, a weight of scoring is defined: 1x goblet cell loss; 2x crypt loss; 2x hyperplasia; and 3x submucosal inflammation. Thus, the total histological scoring was the combined score [0–22].[33] For quantification of CD3, sections were stained as described in the in situ section and analysed using ImageJ software [rsbweb.nih.gov/ij/; National Institutes of Health, Bethesda, MD].

2.5. Endoscopy

Endoscopy of the colon was performed under anaesthesia with 3% isoflurane/O_2. The Olympus URF type V endoscope [Zoeterwoude, The Netherlands] was rectally inserted for a maximum of 10 cm and videos of the endoscopy were recorded using a Medicap USB200 Medical Digital Video Recorder [Roermond, The Netherlands], while retracting the endoscope. A blinded and trained technician determined the adjusted murine endoscopic index of colitis severity [MCEIS].[33] The score consisted of the following items: wall thickening, vascularity, and granularity. Wall thickening: grade 0: transparent, 1: moderate, 2: marked, 3: non-transparent. Vascularity: grade 0: normal, 1: moderate, 2: marked, 3: bleeding. Granularity: grade 0: none, 1: moderate, 2: marked, 3: extreme. This determined a total endoscopy score ranging from 0 to 9.

2.6. Flow cytometry

For *ex vivo* flow cytometry, spleen and mesenteric lymph nodes were collected in phosphate-buffered saline [PBS] on ice. Tissue was mashed and flushed through a cell strainer. Splenic cells were separated from erythrocytes by incubation of the cell suspension in ery-lysis buffer on ice for 3 min. Cells were resuspended in RPMI medium. To assess activation markers in splenic T cells, we stained for αCD3 [clone 17A2, PerCPeFluor 710, eBioscience, San Diego, CA], αCD4 [clone RM 4–5, BV605, BD Pharmingen, Vianen, The Netherlands], αCD25-PECy7 [clone PC61, BD Pharmingen], and αCD69-BV605 [clone H1.2F3; BD Horizon]. For analysis of colonic tissue, colons were removed and longitudinally cut in half. One part was used for flow cytometry, the other for histological assessment. Colon sections were cut into 0.5-cm pieces and washed three times in PBS. Subsequently, tissue sections were incubated in Hank's balanced salt solution [HBSS; Thermofisher Scientific] supplemented with 5 mM EDTA for 20 min while shaking. Cells and remaining tissue were pelleted, minced very fine, and incubated in HBSS supplemented with 2% FBS, 60 mg/mL Liberase TL, and 40 mg/mL DNAse [both Sigma Aldrich] for 40 min while stirring. Suspensions were then passed through a 100-mm cell strainer, pelleted, and stained. For intracellular staining of cytokines IFNγ, IL4, and IL17, T cells were stimulated with 50 ng/ml phorbol myristate acetate [PMA, Sigma] and 1000 ng/ml ionomycin [Sigma]. After 2 h, 10 µg/ml Brefeldin A [Sigma] was added and cells were incubated for 4 additional hours. Cells were washed in PBS and stained for a LiveDead cell viability marker [LifeScience, Amsterdam, The Netherlands]. After washing with PBS-EDTA, cells were stained for αCD3- PerCPeFluor 710 [clone 17A2, eBioscience] and αCD4-BV605 [clone RM4-5, BD Horizon] before being fixed in 1% paraformaldehyde [Merck, Kenilworth, NJ]. Cells were stained for αIL4-PE [clone 11B11, BD Pharmingen], αIFNγ-APC [clone XMG1.2, BD Horizon], and αIL17-FITC [clone TC11-18H10, BD Pharmingen], all dissolved in 0.5% saponin buffer [Sigma] to permeabilise the cells. Antibodies used for the macrophage panel were obtained from Biolegend [San Diego, CA]: αCD45-APC-Cy7 [clone 30-F11], αCD11b-PerCP [clone M1/70], αCD64-PE [clone X54–5/7.1], αCD206-AF488 [clone MR5D3]; from BD Bioscience Vianen, The Netherlands: αLy6G-AF700 [clone 1A8]; and from eBioscience: αLy6C-APC [clone HK1.4]. Cells were stained using DAPI to discriminate live cells. Cells were analysed using a FACS Fortessa [BD Biosciences] and analysed using FlowJo software [Treestar Inc., Ashland, OR].

2.7. Lymphocyte proliferation assays

Murine splenic T cells were collected as described above and plated out in triplicate in a 96-well plate containing 100 000 cells per well. Cells were stimulated with either PMA [50 ng/ml] combined with ionomycin [1000 ng/ml] or with αCD3/CD28 beads [Thermofisher Scientific, 10 splenocytes/bead] in presence or absence of TYK2i [10-10 000 nM]. After 2 days, tritium [^{3}H; Sigma] was added [0.3 µl/50 µl]. After a further 24 h, cells were harvested using the Cell Harvester [Perkin Elmer, Waltham, MA, USA]. In human T cell activity assays, lymphocyte proliferation was induced either by adding αCD3/CD28 beads [10 lymphocytes/bead] or by mixing peripheral blood mononuclear cells [PBMCs] in a 1 to 1 ratio [mixed lymphocyte reaction], in presence or absence of TYK2i [10-10 000 nM]. To determine human lymphocyte viability in presence of TYK2i, lymphocytes were treated with TYK2i [2000 nM] or DMSO for 24 h . Next, cell viability was verified using Vi-CELL XR Cell Viability Analyser [Beckman Coulter], based on trypan blue dye exclusion method in accordance with the manufacturer's instructions. The viability was reported as percentage live cells.

2.8. In situ hybridisation

Sections were cut freshly and dried overnight at 37°C. In situ hybridisation was performed using the RNAscope Reagent 2.5HD Red system [Advanced Cell Diagnostics, Milan, Italy], using probes Mm-IFNγ and Mm-IL17A. After development of the FastRed signalling, sections were washed once in tapwater and twice in PBS. Sections were then stained using αCD3 [Dako, Copenhagen, Denmark] and Goat-αRabbit-AF488 [Invitrogen], mounted in SlowFade Gold containing DAPI [Invitrogen], and imaged using a Leica DM6000B microscope. Images were analysed using ImageJ software. CD3-positive staining was calculated relative to DAPI staining. T cell skewing was calculated as the number of $CD3^+$ cells

containing at least two positive spots, as per the manufacturer's instructions.

2.9. Quantitative real-time polymerase chain reaction

Isolation of mRNA from colon sections was performed using Tri Reagent and the Bioline ISOLATE II RNA mini kit [GC biotech B.V., Alphen a/d Rijn, The Netherlands]. RNA concentration was measured using the Nanodrop ND-1000 spectrophotometer [Nanodrop Technologies, Wilmington, DE, USA]. cDNA was synthesised using the Revertaid first strand cDNA synthesis kit [Fermentas, St. Leon-Rot, Germany]. We conducted a quantitative polymerase chain reaction [PCR] using SensiFAST SYBR No-ROX [GC Biotech B.V.] on a LightCycler 480 [Roche Applied Science] and analysed expression levels of genes listed in Table 1 in mRNA obtained from either murine colon sections or human T cells.

2.10. Human dendritic cell-T cell co-culture assays

Peripheral blood mononuclear cells [PBMCs] were obtained from three healthy human donors [Sanquin, Amsterdam, The Netherlands] after Ficoll paque PLUS [GE-Healthcare, Hoevelaken, The Netherlands] density centrifugation. Next, monocytes were isolated using $CD14^+$ MACS selection kit [Miltenyi Biotech, Leiden, The Netherlands]. The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an ethical committee-approved protocol. Monocytes were pelleted and resuspended at a final concentration of 1 x 10^6 cells/ml in media [RPMI 1640 + 5% FBS + 1% 100 U/mL Pen Strep + 1% 2 mM L-Glut] containing 30 ng/ml granulocyte monocyte colony stimulating factor [GM-CSF; R&D, Abingdon, UK] and 20 ng/ml IL4 [R&D] for 5 days to achieve dendritic cell [DC] polarisation. From the same PBMC fraction, T cells were obtained using a CD4 Isolation kit [Miltenyi Biotech]. The cell pellets were resuspended in 40 ml medium [RPMI 1640 + 10% FBS + 1% 100 U/mL Pen Strep + 1% 2 mM L-Glut] containing 10 ng/ml IL-7 [R&D] and placed in the incubator at 37°C, 5% CO_2, for 5 days until co-culture. Before co-culture, DCs were washed and incubated with either medium or with medium containing lipopolysaccharide [LPS] and Revaxis [Sanofi Pasteur MSD Limited] for 24 h or for 48 h for protein analyses, followed by washing. T cells were washed and incubated with either DMSO or TYK2i [2000 nM] for 24 h, followed by washing. Next DCs, incubated with either medium or LPS/Revaxis, were co-cultured with T cells treated with either DMSO or TYK2i, resulting in four combinations of co-culture. After 24 and 48 h, supernatant was collected for the protein level measurement of IFNy [Th1 marker], IL13 [Th2 marker], IL17A [Th17 marker], and IL10 [T_{reg} marker]. For transcriptional analyses, DCs were incubated with LPS and Revaxis for 6 h and for 20 h, and co-cultured with T cells. We measured markers of Th1 [TBX21; T-Box Transcription Factor TBX21], Th2 [GATA3], Th17 [RORC], and T_{reg} [FoxP3] on mRNA level in T cells. Analysis and extraction of data was performed by using MSD Sector Imager 6000 Reader [Biomax 800325] and Discovery workbench software v4.0.11.

2.11. Human ex vivo assays

Biopsies were obtained from resection specimens from three Crohn's disease patients and three ulcerative colitis patients, using standard-size [2.2-mm outer diameter] spiked double-bite biopsy forceps. Biopsies were incubated in culture medium (RPMI 1640 containing 10% fetal calf serum [Lonza], 2 mM l-glutamine [Invitrogen], 100 U/mL penicillin-streptomycin [Invitrogen], 50 µg/mL gentamicin [Lonza], and 50 µg/mL amphotericin B [Thermo Fisher]) in the presence of TYK2i [2000nM] or vehicle [DMSO;Sigma] for 24 h, and supernatant was collected for analysis. Levels of TNFα, IL6, IFNγ, and IL17 were determined using a cytometric bead array [BD CBA Human Inflammatory Cytokines Kit, BD Biosciences, San Jose, CA]. The human biopsy samples were obtained under an ethical committee-approved protocol and all patients gave informed consent.

2.12. Statistical analysis

Statistical analysis was performed using GraphPad Prism 7.0 [GraphPad Software, La Jolla, CA]. For analysis between two groups, non-parametric data were subjected to a Mann-Whitney U test and parametric data were subjected to a t test. For multigroup analysis, non-parametric data were subjected to one-way analysis of variance [ANOVA] [Kruskall-Wallis] followed by Dunn's post hoc analysis; a *p*-value [*p*] <0.05 was considered significant.

3. Results

3.1. TYK2 inhibitor reduces disease in T cell transfer colitis

TYK2 inhibition was studied *in vivo* using an orally delivered TYK2i in the T cell transfer model of colitis. Treatment was started after colitis onset and was confirmed by endoscopy at 37 days after start of the transfer. Daily gavage with 10, 30 or 70 mg/kg TYK2i was administered from Day 38 until sacrifice on Day 63. All compound doses tested were well tolerated, and the 70-mg/kg dose impaired the weight loss characteristic of the model in treated animals at sacrifice [Figure 1a]. Treatment with TYK2i significantly decreased the DAI at the highest dose given [Figure 1b]. Also splenic weight, a general marker for inflammation, was significantly decreased at the highest dose given [Figure 1c]. The colon weight-length ratio was not affected by the treatment [Figure 1d]. At sacrifice, the 70-mg/kg dose of TYK2i significantly reduced the endoscopic disease severity [Figure 1e]. An example of the endoscopic image revealed that mice treated with 70 mg/kg TYK2i initially develop oedema, fibrin deposits, and a loss of vascular pattern visible at Day 51. However, at Day 63, the endoscopic image of the TYK2i-treated animals revealed a healthy appearance of the mucosa with a normal vascular pattern, whereas the endoscopic image of vehicle-treated mice worsened at Day 63 [Figure 1f]. Histological scoring, the primary readout for colitis in this model, was reduced in a dose-dependent manner by TYK2i [Figure 1g]. Colon sections of vehicle-treated mice displayed clear crypt and goblet cell loss, crypt hyperplasia, and infiltration of immune cells, whereas TYK2i-treated mice from the 70-mg/kg treatment group were almost indistinguishable from those from the non-transferred healthy mice [Figure 1h]. Expression analyses of cytokines generally involved in T cell effector function in whole colon tissue showed that treatment with the highest dose of TYK2i attenuated the induction of IFNγ transcripts while leading to enhanced expression of IL17 and IL23a [IL23p19]. No changes in mRNA expression of IL12a [IL12p35], IL12b [IL12p40], and IL22 were observed after treatment with TYK2i [Supplementary Figure 1A, available as Supplementary data at *ECCO-JCC* online]. TYK2i blunted induction of the IFN-induced gene CCL5, but not CXCL10 [Supplementary Figure 1B]. Last, we measured mRNA expression of Th hallmark transcription factors TBX21, GATA3, RORC, and

Table 1. Primer sequences.

Gene	Abbreviation	Species	Forward sequence	Reverse sequence
ßeta actin	ßactin	Murine	TTCTTTGCAGCTCCTTCGTT	ATGGAGGGGAATACAGCCC
Hypoxanthine guanine phosphoribosyl transferase	HPRT	Murine	CCTAAGATGAGCGCAAGTTGAA	CCACAGGACTAGAACACCTGCTAA
Interferon gamma	IFNγ	Murine	TACTACCTTCTTCAGCAACAGC	AATCAGCAGCGACTCCTTTTC
Interleukin 12p35	IL12p35	Murine	ATGACCCTGTGCCTTGGTAG	TCTCCCACAGCAGGTTTCTG
Interleukin 12p40	IL12p40	Murine	AGACCCTGCCCATTGAACTG	CGGGTCTGGTTTGATGATGTC
Interleukin 17	IL17	Murine	AAAGCTCAGCGTGTCCAAACA	CTTCATTGCGGTGGAGAGTC
Interleukin 22	IL22	Murine	CGGCTCATCGGGGAGAAAC	TGACTGGGGGAGCAGAACG
Interleukin 23p19	IL23p19	Murine	GACCCACAAGGACTCAAGGA	ACTAGAAGTCAGGCTGGGCA
C-C motif chemokine ligand 5	CCL5	Murine	CTGCTGCTTTGCCTACCTCT	CACTTCTTCTCTGGGTTGGC
C-X-C motif chemokine ligand 10	CXCL10	Murine	CGTCATTTTCTGCCTCATCC	CCTATGGCCCTCATTCTCAC
T-box 21 [TBX21]	TBet	Murine	GGTGTCTGGGAAGCTGAGAG	GAAGGACAGGAATGGGAACA
GATA-binding protein 3	GATA3	Murine	CTTATCAAGCCCAAGCGAAG	CATTAGCGTTCCTCCTCCAG
RAR-related orphan receptor C	Rorγ	Murine	GGTGATAACCCCGTAGTGGA	CTGCAAAGAAGACCCACACC
Forkhead box P3	FoxP3	Murine	TGGAGCTGGAAAAGGAGAAG	TACTGGTGGCTACGATGCAG
Interleukin 10	IL10	Murine	TGTCAAATTCATTCATGGCCT	ATCGATTTCTCCCCTGTGAA
Interleukin 6	IL6	Murine	AGTTGCCTTCTTGGGACTGA	TCCACGATTTCCCAGAGAAC
Interferon-induced protein 44	Ifi44	Murine	CTGATTACAAAACAAGACAT GACAGAC	AGGCAAAACCAAAGACTCCA
Oligoadenylate synthetase like 2	Oasl2	Murine	GACTGATGACATTATTTGGA TGGA	GCTGGTTTTGAGTCTCTGGAA
T-box 21 [TBX21]	TBet	Human	GGTTGCGGAGACATGCTGA	GTAGGCGTAGGCTCCAAGG
GATA-binding protein 3	GATA3	Human	GCCCCTCATTAAGCCCAAG	TTGTGGTGGTCTGACAGTTCG
RAR-related orphan receptor C	Rorγ	Human	GTGGGGACAAGTCGTCTGG	AGTGCTGGCATCGGTTTCG
Forkhead box P3	FoxP3	Human	GTGGCCCGGATGTGAGAAG	GGAGCCCTTGTCGGATGATG
ßeta actin	ßactin	Human	GCTGTCCACCTTCCAGCAGA	CGCCTAGAAGCATTTGCGGT

FoxP3 [Supplementary Figure 1C]. Treatment with 70 mg/kg TYK2i elevated RORC expression compared with vehicle [Supplementary Figure 1C] Transcription of IL6 and IL10 expression was not significantly affected by TYK2i treatment in whole colon [Supplementary Figure 1D].

3.2. TYK2 kinase activation drives T cell transfer-induced colitis

To confirm that the improved colitis was due to inhibition of T cell derived TYK2 signalling and not due to off-target effects, we crossed RAG1$^{-/-}$ animals with animals carrying a point mutation in the ATP-binding pocket of the TYK2 kinase domain [TYK2^{KE}].[29] To test the model in absence of all TYK2-mediated kinase activity, these RAG1$^{-/-}$TYK2^{KE} mice were transferred with TYK2^{KE}-CD45RB^{high} T cells [TYK2^{KE} >TYK2^{KE}] and compared with RAG1$^{-/-}$ animals receiving wild type [WT]-CD45RB^{high} T cells [WT >RAG1]. Disease onset was confirmed by endoscopy at Day 37. In TYK2^{KE} recipient mice receiving TYK2^{KE} T cells, weight loss was reduced as compared t with RAG1$^{-/-}$ mice transferred with WT-CD45RB^{high} T cells [Figure 2a]. DAI, colon weight-length ratio, and splenic weight were significantly decreased in the TYK2 inactive mice as compared with WT-CD45RB^{high}-transferred RAG1$^{-/-}$ mice [Figure 2b-d]. Endoscopic scores were significantly decreased in TYK2^{KE}-transferred mice as compared with the WT-transferred controls [Figure 2e-f]. Histopathology scores were significantly lower in TYK2^{KE}-transferred mice as compared with WT-transferred RAG1$^{-/-}$ mice [Figure 2g]. This was in good alignment with the apparent crypt and goblet cell loss, crypt hyperplasia, and infiltration of immune cells, observed in the WT-transferred RAG1$^{-/-}$ mice, and the absence of these histological abnormalities in the TYK2^{KE}-transferred animals [Figure 2h].

3.3. The protective effect of TYK2 kinase inhibition is mediated through T cells

Next, we aimed to delineate the contribution of TYK2 kinase activity in either lymphoid or non-lymphoid cells within the T cell transfer colitis model. To this end, we performed two separate T cell transfer experiments. In the first experiment we transferred RAG1$^{-/-}$ animals with either TYK2^{KE}-CD45RB^{high} T cells [TYK2^{KE} >RAG1] or WT-CD45RB^{high} T cells [WT >RAG1] to assess the contribution of TYK2 in T cells in the disease process. In the second experiment, we investigated the contribution of non-lymphoid cells, by transferring WT-CD45RB^{high} T cells into RAG1$^{-/-}$TYK2^{KE} recipient mice [WT >TYK2^{KE}], so these animals lacked TYK2 kinase activity in all non-T cells, and comparing this with WT-CD45RB^{high} T cells transferred into RAG1$^{-/-}$ mice [WT >RAG1]. When TYK2 kinase activity was abrogated in CD45RB^{high} T cells, weight loss was reduced in recipient RAG1 animals [Figure 3a; left]. However, upon transfer of WT-CD45RB^{high} T cells in RAG1$^{-/-}$TYK2^{KE} animals, we observed a regular decrease in weight indifferent from control colitis [Figure 3a; right]. Transfer of TYK2^{KE}-CD45RB^{high} T cells in RAG1$^{-/-}$ recipient animals resulted in a significantly decreased DAI [Figure 3b; left], whereas in the RAG1$^{-/-}$TYK2^{KE} animals transferred with WT-CD45RB^{high} T cells, the DAI was not significantly decreased [Figure 3b; right]. In both experiments, splenic weight was not significantly decreased [Figure 3c]. The colon weight-length ratio was decreased in animals receiving T cells with abrogated TYK2 kinase activity, whereas this ratio remained unaffected upon transfer of WT-CD45RB^{high} T cells in RAG1$^{-/-}$TYK2^{KE} recipient animals [Figure 3d]. The endoscopic severity significantly decreased upon transfer with TYK2^{KE}-CD45RB^{high} T cells in RAG1$^{-/-}$ mice [Figure 3e left; upper Figure 3f], whereas upon transfer of WT-CD45RB^{high} T cells in RAG1$^{-/-}$TYK2^{KE} recipient animals, severity was essentially

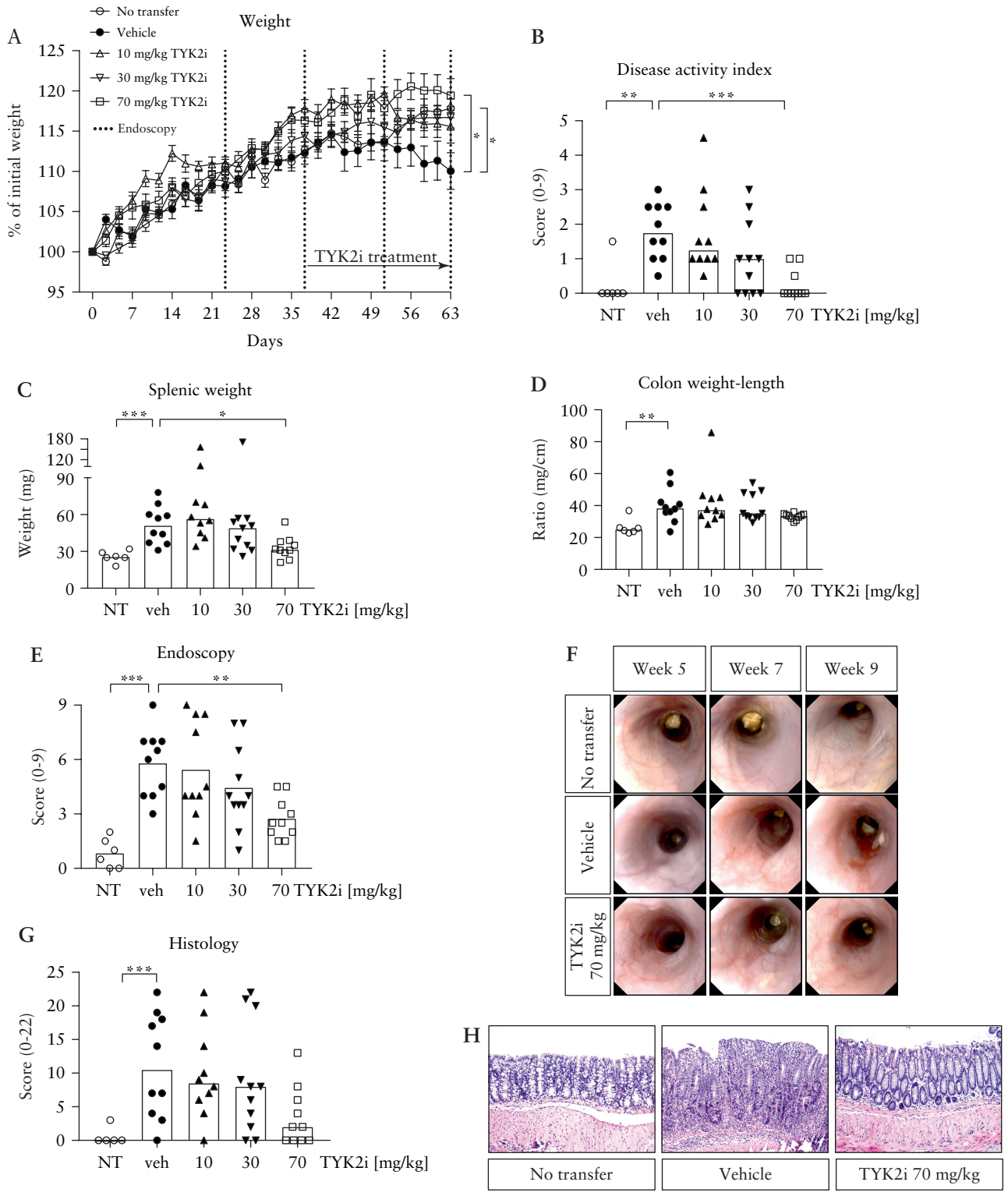

Figure 1. Oral tyrosine kinase 2 inhibitor reduces disease in T cell transfer colitis. A] Weight curves, expressed as percentage of initial body weight. Data points are expressed as mean with standard error of the mean [SEM]. Animals received daily oral gavage from Day 38 until sacrifice [Day 63] with either vehicle [veh] or 10, 30, or 70 mg/kg of the tyrosine kinase 2 inhibitor [TYK2i]. A non-transferred control group was included [NT]. Symbols indicate statistical significance of NT versus veh and veh versus 70 mg/kg groups, respectively. B] The disease activity index [DAI] was scored at sacrifice [Day 63]. C] Splenic weight at sacrifice expressed in milligrams. D] Colon weight-length ratio at sacrifice expressed as mg/cm. E] The endoscopy score at day of sacrifice. F] Representative endoscopy image comparing NT, vehicle, and TYK2i-treated animals. G] The histology score at sacrifice. H] Representative picture of a haematoxylin and eosin [HE] staining of the distal colon comparing NT, vehicle, and TYK2i-treated animals; 100 × magnification. All bar graphs B-G: data are expressed as median with individual data points representing individual animals. Statistical analyses A-G: Mann-Whitney U test [NT vs vehicle] and Kruskal-Wallis test followed by post hoc Dunn's test [veh vs 10, 30, or 70 mg/kg]; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

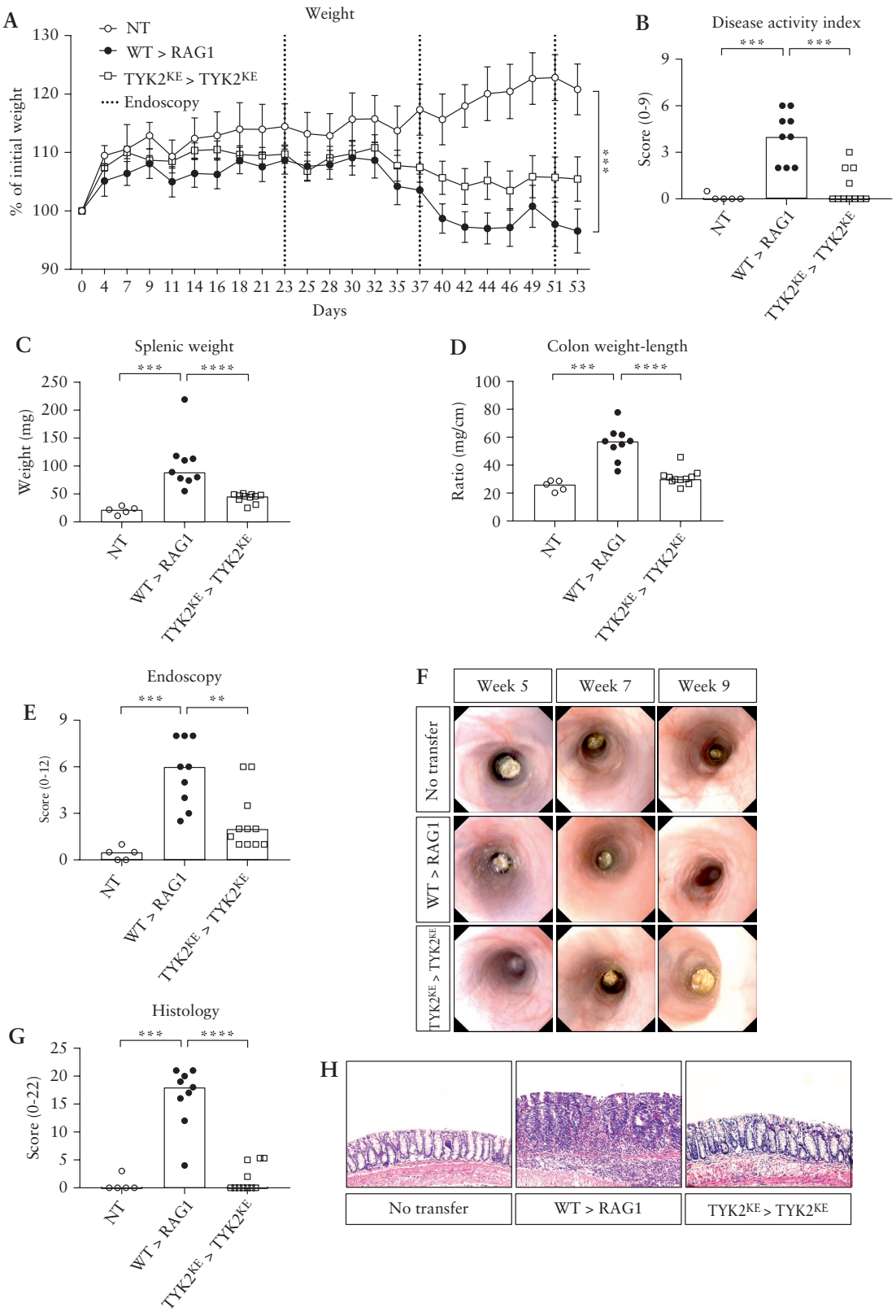

Figure 2. Tyrosine kinase 2 [TYK2] kinase activation drives T cell transfer induced colitis. A] Weight curves, expressed as percentage of initial body weight. Data points are expressed as mean with standard error of the mean [SEM]. RAG1$^{-/-}$ animals were transferred with wild type [WT] T cells [WT >RAG1]; RAG1$^{-/-}$ TYK2^{KE} animals were transferred with T cells lacking TYK2 kinase activity [TYK2^{KE}>TYK2^{KE}]. A non-transferred control group was included [NT]. B] The disease activity index [DAI] was scored at sacrifice [Day 53]. C] Splenic weight at sacrifice expressed in milligrams. D] Colon weight-length ratio at sacrifice expressed as mg/cm. E] The endoscopy score at day of sacrifice. F. Representative endoscopy image comparing NT-, WT-, and TYK2^{KE}-transferred animals. G] The histology score at sacrifice. H] Representative picture of a haematoxylin and eosin [HE] staining of the distal colon comparing NT-, WT-, and TYK2^{KE}-transferred animals; 100 x magnification. All bar graphs, B-G: data are expressed as median with individual data points. Statistical analyses A-G: Mann-Whitney U test; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$; $^{****}p \leq 0.0001$.

unaffected [Figure 3e right; lower Figure 3f]. Histology scores indicated amelioration of colitis in RAG1$^{-/-}$ animals transferred with TYK2^{KE} T cells, compared with recipient RAG1$^{-/-}$TYK2^{KE} mice transferred with WT-CD45RB^{high} T cells [Figure 3g-h]. Together, this demonstrates that the colitogenic potential of transferred T cells is mediated through TYK2 signalling in T cells rather than through TYK2 signalling in non-lymphoid cells.

To evaluate whether lack of TYK2 signalling might have resulted in decreased T cell engraftment, thus affecting colitis development, we measured the proportion of live CD3^{+} T cells in spleen and mesenteric lymph nodes. No differences in percentage of CD3 + T cell homing between WT and TYK2^{KE} transferred animals were observed, making it unlikely that a difference in colitis scores was due to failure of TYK2-deficient T cells to engraft in the recipient animals [Supplementary Figure 2a, available as Supplementary data at *ECCO-JCC* online].

3.4. TYK2 inhibition interferes with T cell skewing

Next, we investigated whether TYK2 inhibition ameliorates colitis via the inhibition of differentiation of colitogenic effector T cell populations. We assessed tissue T cell populations during colitis and in the presence of the TYK2i. In colon sections of TYK2i-treated mice, we observed a decreased number CD3^{+} T cells as compared with vehicle-treated animals, in line with the general histopathological scores [Figure 4a]. Similar data were observed in animals lacking TYK2 kinase activity in the transferred T cells [Figure 4b]. In situ hybridisation revealed that upon inhibition of TYK2, the remaining T cells expressed lower levels of IFNγ [Figure 4c]. Next, we analysed colonic IFNγ-, IL17-, IFNγ/IL17- [double positive], and IL4-expressing T cells ex vivo during colitis by flow cytometry. In RAG1$^{-/-}$TYK2^{KE} mice transferred with TYK2^{KE}-CD45RB^{high} T cells, the percentage of IFNγ-expressing T cells was decreased as compared with animals transferred with WT-CD45RB^{high} T cells. Both the percentage of IL17-expressing T cells and that of IFNγ/IL17 double-expressing T cells were increased as compared with the WT-transferred animals, whereas IL4-expressing T cells were unaffected [Figure 4d]. Next, we performed expression analyses on whole colon tissue obtained from the two separate T cell transfer experiments. We observed that IFNγ mRNA expression was increased in WT-transferred RAG1$^{-/-}$ hosts, whereas this increase was reduced in TYK2^{KE}-transferred RAG1$^{-/-}$ hosts[Supplementary Figure 2b, left graph]. In the subsequent transfer experiment, a similar IFNγ mRNA expression pattern was observed in the experimental group lacking TYK2^{KE} signalling [TYK2^{KE} > TYK2^{KE}], whereas the in WT-transferred TYK2^{KE} host [WT >TYK2^{KE}] IFNγ was not significantly decreased [Supplementary Figure 2b, right graph]. Notably, a reduced IL17 mRNA expression in whole colon tissue was observed in both experiments as described above, likely reflecting the overall decreased presence of CD3 + cells [Supplementary Figure 2b]. In mice lacking TYK2 signalling in both experiments, mRNA expression of CXCL10 was decreased, whereas IL10 and IL6 mRNA expressions were unaffected [Supplementary Figure 2b].

As TYK2 is known to affect interferon type I as well as type II IFN signalling, we investigated IFN type I induced genes ifi44 and oasl2 in colon homogenates and found expression being attenuated in TYK2^{KE}-transferred homogenates, suggesting a decreased type I interferon signalling [Supplementary Figure 2c]. Additional analyses on transcription factors TBX21, GATA3, RORC, and FoxP3 showed transcription similar to that upon pharmacological TYK2 inhibition [Supplementary Figure 3a, available as Supplementary data at *ECCO-JCC* online]]. In RAG1$^{-/-}$TYK2^{KE} recipients receiving WT-CD45RB^{high} T cells, we observed no significant changes in percentages of IFNγ-, IL17-, IFNγ/IL17-, or IL4-expressing T cells as measured by flow cytometry [Figure 4e, gating strategy Supplementary Figure 3b]

To investigate the role of the different T cell transfer experiments on myeloid populations in inflamed colon mucosa, we measured populations of M1-like and M2-like macrophages by flow cytometry. Colonic myeloid cells were gated as DAPI^{-}CD45^{+}Ly6G^{-}CD11b^{+} cells and subdivided into regulatory [CD206^{hi}Ly6C^{lo}] M2-like macrophages and inflammatory [CD206^{lo}Ly6C^{hi}] M1-like macrophages. However, we did not observe any changes in distribution of the macrophage markers Ly6C [M1] and CD206 [M2] [Figure 4f].

To assess T cell proliferation and activation, we performed further analyses of *ex vivo* splenic lymphocytes. We observed that CD69, a marker for T cell activation, was decreased in the TYK2^{KE} T cell transferred mice. In contrast, CD25, required for induction of T cell proliferation via IL2, was unaffected [Supplementary Figure 4a]. In addition, proliferation of *ex vivo* obtained splenic lymphocytes was not affected in TYK2^{KE} T cells obtained after transfer when compared with WT T cells, as measured by a tritium incorporation assay [Supplementary Figure 4b]. Next, we tested proliferation directly *in vitro* using two different methods of proliferation [αCD3/CD28; PMA/ionomycin] in murine splenocytes. Proliferation was not affected in the presence of high dose TYK2i [8000 nM, Supplementary Figure 4c]. In addition, splenocytes obtained from animals that lack TYK2 kinase activity [TYK2^{KE}] or from animals in which the TYK2 gene is completely disrupted [TYK2^{KO}] did not show abnormalities in proliferation. Consequently, TYK2 signalling did not appear to be a strong contributor to T cell proliferation and we conclude that TYK2i ameliorates colitis through inhibition of cytokine signalling rather than cellular proliferation.

3.5. TYK2 inhibition inhibits the differentiation of human Th1 cells

We also tested the efficacy of TYK2i to interfere with T cell proliferation in human cells. In a viability assay in T cells in presence of varying concentrations of TYK2i, we observed a decreased viability of lymphocytes only at the two highest concentrations [Supplementary Figure 4d]. In T cells isolated from human donors and stimulated with αCD/CD28, we only observed a decreased proliferation at TYK2i concentrations higher than 4000 nM, in line with the viability assay [Supplementary Figure 4e]. In a mixed lymphocyte reaction [MLR], proliferation is induced by co-culture of two different donors. Again, proliferation was significantly decreased only at the highest concentrations of TYK2i [Supplementary Figure 4f].

Next, TYK2i [2000 ng/ml] was tested in a Revaxis-driven autologous DC-T cell assay to assess the effect of TYK2i on proliferation and differentiation of human T cells in an antigen-specific manner. We measured markers of Th1 [TBX21], Th2 [GATA3], Th17 [RORC], and T_{reg} [FoxP3] on mRNA level in T cells. As shown in Figure 5a, mRNA expression of the transcription factor TBX21 was decreased in this assay, whereas GATA3, RORC, and FoxP3 expressions were unaffected. In addition, we measured the secreted protein level of IFNy [Th1 marker], IL13 [Th2 marker], IL17A [Th17 marker], and IL10. In line with the reduced expression of transcription factors, production of IFNγ was decreased at the protein level after 48 h in the presence of TYK2i. Production of IL13, IL17, and IL10 were unaffected [Figure 5b]. We conclude that TYK2i reduces expression of TBX21 in human T cells as in murine cells.

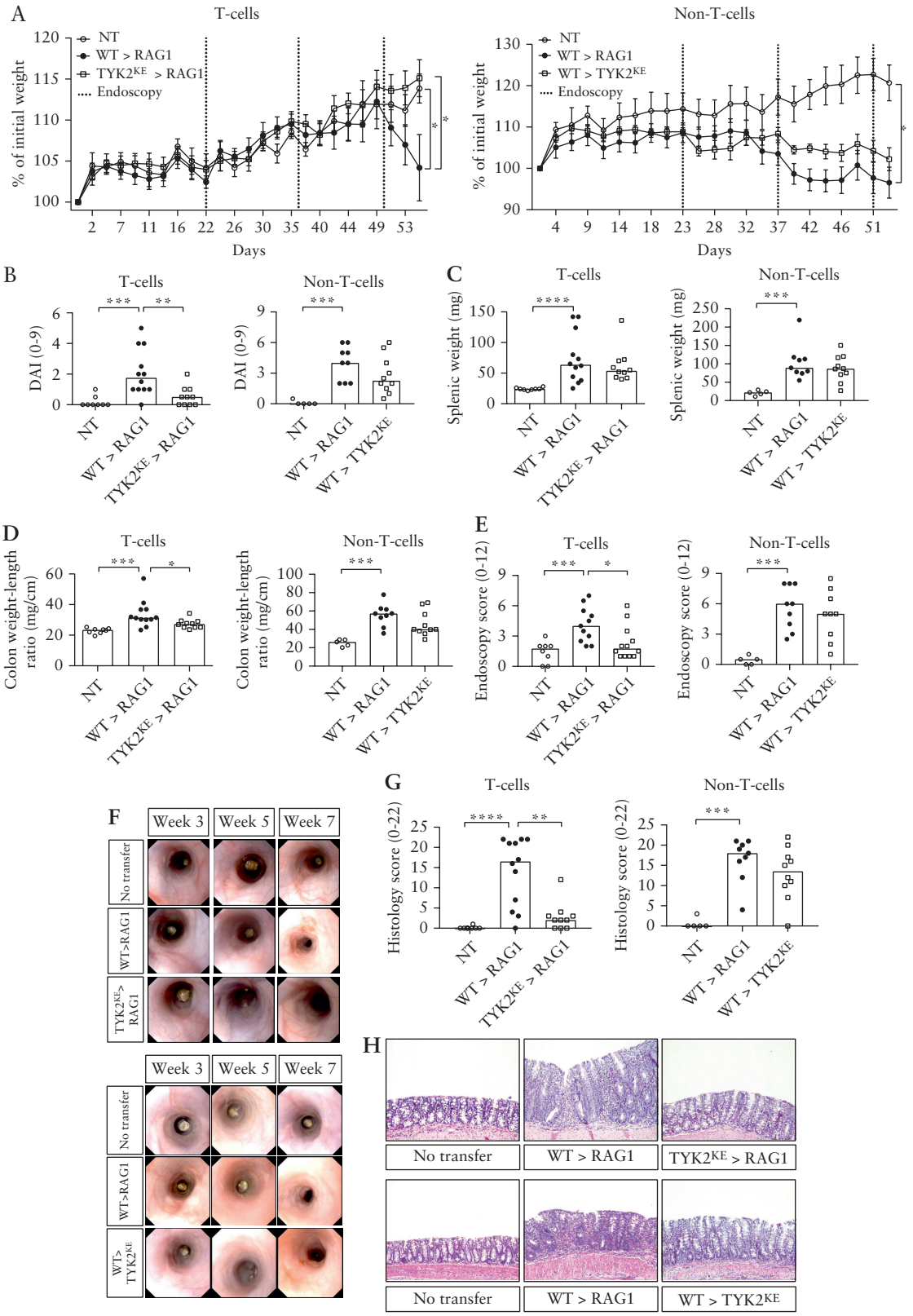

Figure 3. The protective effect of TYK2 kinase inhibition is mediated through T cells. A] Weight curves, expressed as percentage of initial bodyweight. Data points are expressed as mean with standard error of the mean [SEM]. [Left] RAG1$^{-/-}$ animals were transferred with either wild type [WT] T cells [WT >]RAG1] or with T cells lacking tyrosine kinase 2 [TYK2] activity [TYK2^{KE}>RAG1]. [Right] RAG1$^{-/-}$ animals or RAG1$^{-/-}$ back-crossed with TYK2^{KE} animals [RAG1$^{-/-}$TYK2^{KE} animals] transferred with WT T cells [respectively WT >RAG1 or WT >TYK2^{KE}]. A non-transferred control group was included [NT] in both experiments. B] The disease activity index [DAI] was scored at sacrifice [Day 56 T cell; Day 53 non-T cell] C] Splenic weight at sacrifice expressed in milligrams. D] Colon weight-length ratio at sacrifice expressed in mg/per cm. E] The endoscopy score. F] Representative endoscopy image comparing NT, RAG1, and TYK2^{KE} animals after transfer of WT T cells. G] The histology score at sacrifice. H] Representative picture of a haematoxylin and eosin [HE] staining of the distal colon comparing NT, RAG1, and TYK2^{KE} animals after transfer of WT T cells; 100 × magnification. All bar graphs B-G: data are expressed as median with individual data points. Statistical analyses A-G: Mann-Whitney U test; *$p \leq 0.1$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$.

Finally, we investigated the effect of TYK2i on inflamed human UC and CD biopsy explants. In CD donors as well as in the UC donors, we observed a decrease in secretion of TNFα, IL6, and IL17 [albeit to a lesser extent], in presence of TYK2i as compared with vehicle. The IFNγ concentration decreased in presence of TYK2i; however, the IFNγ concentration measured in the biopsy explants was below the detection level. Although a trend towards a decreased secretion was apparent, none of these changes in protein level was significant, because of high variation among tissues [Figure 5c].

4. Discussion

A growing interest for selectively targeting JAK kinases has emerged, following the demonstrated efficacy of tofacitinib in the treatment of immune-mediated diseases including IBD, psoriasis, and rheumatoid arthritis [RA].[5,7,34–36] After selective targeting JAK1, attention is now being focused on TYK2, with an ongoing phase III trial investigating the TYK2 selective inhibitor BMS-986165 in psoriasis [clinicaltrial.gov NCT03611751.] TYK2 phosphorylation is essential in the signalling of a number of cytokines involved in the pathogenesis of IBD, in particular IL12 and IL23, but affects a wider range of cytokines including type 1 IFN, IL6, IL10, IL13, IL22.

We demonstrate here that T cells lacking TYK2 kinase activity failed to induce colitis. *In vitro* and *ex vivo*, both Th1 and Th17 cell differentiations from naïve $CD4^+$ cells are affected in $TYK2^{KE}$ mutant mice, confirming earlier studies describing the potential of TYK2 inhibition to differentially influence Th1/Th17 differentiation animal models for autoimmune diseases, including RA, psoriasis,[28] and colitis.[17,37] Our data support earlier studies demonstrating efficacy of TYK2 inhibition in DSS- and TNBS-induced colitis models in TYK2-deficient mice.[17] In both models, TYK2-deficient animals displayed a slower disease onset, without affecting weight loss, and a less severe shortening of the colon. In addition, the mRNA expression level of IFNγ, IL17, IL12, and IL23 was decreased in colon tissue obtained from TYK2-deficient mice. In contrast, others have shown that Tyk2 deficiency is associated with an altered composition of the gut microbiota and aggravation of experimental colitis. In TYK2-deficient mice, reduced phospho-STAT3 resulted in inefficient proliferation of intestinal epithelial cells. These discrepancies may be explained by varying susceptibility to DSS of the different animal strains. This cell-type specific effect of TYK2 inhibition appears to be detrimental in our T cell mediated colitis model, as the net effect of TYK inhibition alleviated colitis, but may be relevant in other types of experimental colitis.[38] The role of TYK2 in human disease was suggested when polymorphisms in TYK2 were associated with development of Crohn's disease, in potential combination with polymorphisms in the STAT3 gene.[39,40]

JAK inhibitors have been used widely in inhibition of inflammatory activity in IBD. A recent study showed inhibition of colitis using a an alternative TYK2 inhibitor, BMS-986165, that specifically targets the pseudo kinase domain of TYK2 rather than the catalytic domain, allowing a better specificity.[41,42] This study used a prophylactic set-up with administration of the inhibitor before disease onset.[42] In the current study, we confirmed the onset of colitis by endoscopy before initiation of treatment, allowing for evaluation of a truly therapeutic effect. Both studies clearly support the potential for TYK2 inhibition in colitis. Notably, although the TYK2 inhibitor has good selectivity versus TYK2 over JAK1, 2, and 3 in ATP-binding assays[25] and in its ability to inhibit IL12 and IL23 to activate downstream signalling,[25,2] our study did not demonstrate full selectivity of TYK2 inhibition over other JAK activities in mucosal context. However, T cell transfer studies did support the hypothesis that TYK2i activity exerted colitis protection through reduced TYK2 activation in T cells, adding to the aforementioned activity of the current TYK2i in other *in vivo* models of autoimmune disease and cancer.

A novel TYK2 selective inhibitor, NDI-031301, appears to be effective as an oral treatment in immuno-deficient mice engrafted with T cell acute lymphoblastic leukaemia cells.[21] A TYK2/JAK1 dual oral inhibitor, SAR-20347, was effective in reducing psoriasis-like disease severity and keratinocyte proliferation in an imiquimod-induced psoriasis mouse model. In addition, mRNA expression of IL6, IL17, IL22, IL23, and antimicrobial peptide gene expression were decreased in the skin of SAR-20347 treated animals as compared with vehicle-treated wild type animals and TYK2 mutant mice.[43] Similarly, a TYK2 selective inhibitor, with inhibiting properties equal to our currently used molecule, reduced IL12- and IL23-induced IFNγ and IL17 production, respectively.[28]

We demonstrate that the colitogenic potential of transferred T cells is mediated mainly through TYK2 signalling in T cells rather than through TYK2 signalling in non-lymphoid cells, without affecting T cell proliferation. However, given the wide expression of TYK2, it is likely that other [immune] cells are also affected by TYK2 inhibition. For example, others have investigated TYK2 inhibition in relation to DC-T helper cell differentiation. Upon antigen presentation, DCs produce IL12 and IL23 leading to Th1 and Th17 responses, respectively. In TYK2-deficient DCs, IL12 and IL23 production was decreased, resulting in a decrease in T cell derived IFNγ and increased IL17.[16] In our *in vivo* data, we observed similar effects in the animals lacking TYK2 in non-lymphoid cells, although this did not reach significance. In contrast, in animals lacking TYK2 in the T cell compartment, the effects were considerably more pronounced. Together, this suggests that although the main effect of TYK2i in the current model is driven by T cells, other cells types are clearly affected by lack of TYK2 kinase activity, and the inhibition of IL12/23 signalling leading to altered Th1/Th17 responses is not likely to be the only effector mechanism of TYK2 inhibition.

In a clinical setting, effects on other cell types as well as additional cytokines are relevant from the perspective of side effects. Tofacitinib has been associated with an increase in herpes zoster infections, presumably due to impaired interferon signalling. We also observed decreased expression of type I interferon targets in the animals lacking active TYK2. In a natural setting, several patients carrying null mutations in TYK2 have been described. These patients showed an increased susceptibility to intracellular and viral infections, and impaired but not abolished responses to type I IFN and IL10. Interestingly, all these patients carried mutations resulting in the absence of the TYK2 protein rather than functional mutations in the kinase domain. This is relevant in the light of small molecule inhibitors, as these mainly target the kinase active site but leave the protein itself intact. As TYK2 not only has a catalytic function, but also exerts a scaffolding effect stabilising the receptors for IFN, IL10, and IL12, an actual lack of protein is expected to affect signalling through these pathways more profoundly than mere inhibition of the kinase domain. In a separate publication, a human TYK2-deficient subject was described in whom type III IFN responses and IL12-mediated NK-cell responses were normal despite TYK2 deficiency, leading to a surprisingly mild phenotype.[44] Current clinical trials investigating TYK2 selective inhibitors will hopefully provide more insight in adverse events regarding viral susceptibility.

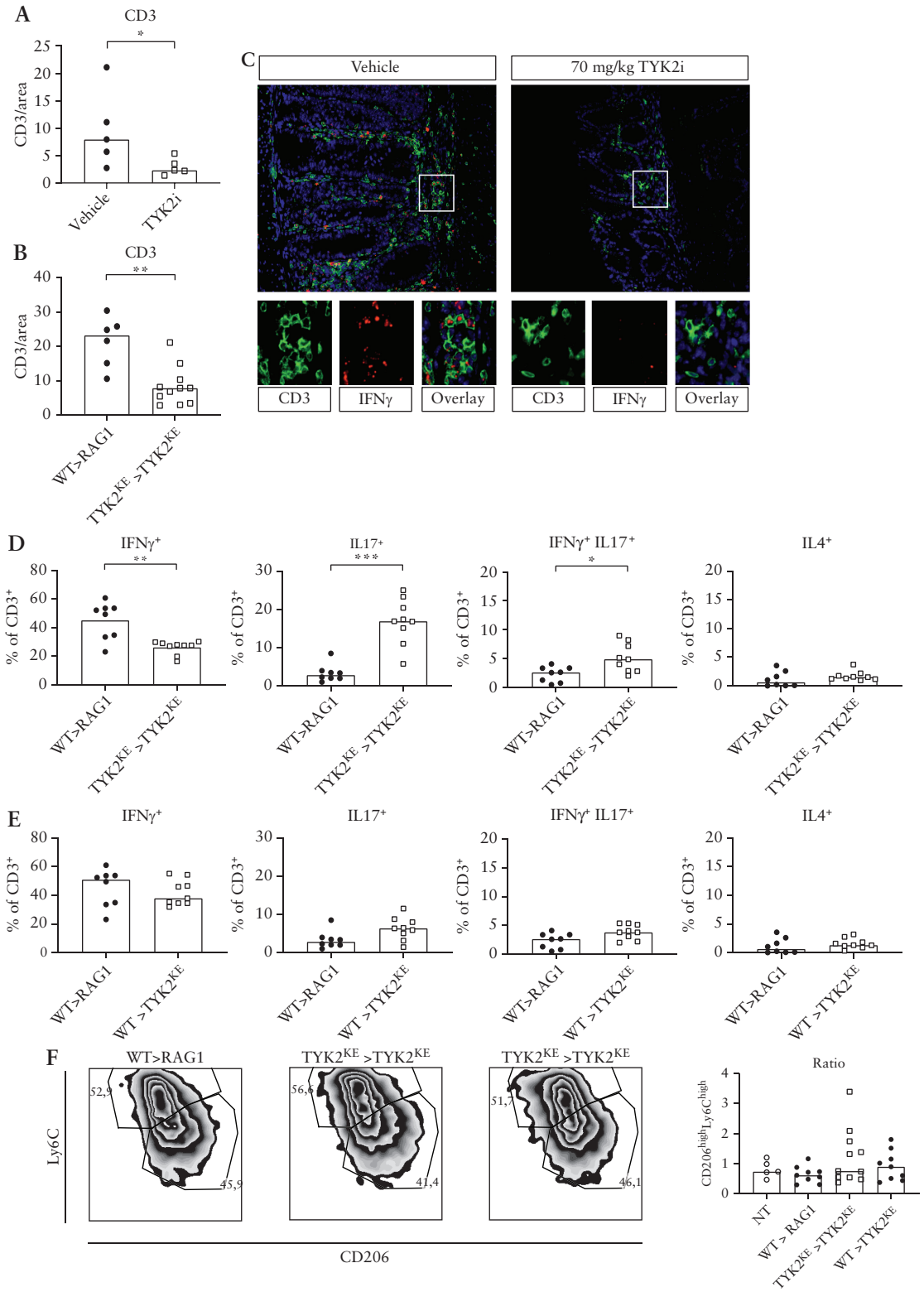

Figure 4. TYK2 inhibition interferes with T cell skewing. A] CD3 expression in colon of RAG1$^{-/-}$ animals transferred with wild type [WT] T cells, treated with vehicle or tyrosine kinase 2 inhibitor [TYK2i]. B] CD3 expression in colon of RAG1$^{-/-}$ animals transferred with WT T cells [WT >RAG1] as compared with RAG1$^{-/-}$TYK2^{KE} recipient animals transferred with TYK2^{KE} T cells [TYK2^{KE}>TYK2^{KE}]. C] Immunofluorescent image of interferon [IFN]γ^{+}CD3^{+} T cells in colon paraffin sections using RNA Scope in animals treated with vehicle or TYK2i 70 mg/kg. CD3^{+} expression is indicated in green, IFNγ mRNA is indicated in red. D] Percentage of IFNγ^{+}-, IL17^{+}-, IFNγ^{+}/IL17^{+}-, and IL4^{+} within the gated CD45^{+}/CD3^{+} T cell population as measured by flow cytometry obtained from whole colon cell suspension in TYK2 animals transferred as in B, as measured by flow cytometry. E] Experiment performed in in D, in RAG1$^{-/-}$ animals transferred with WT T cells [WT >RAG1] as compared with RAG1$^{-/-}$TYK2^{KE} recipient animals transferred with WT T cells [WT >TYK2^{KE}]. F] Colonic myeloid cells were gated as DAPI^{-}CD45^{+}Ly6G^{-}CD11b^{+} cells and subdivided into regulatory [CD206^{hi}Ly6C^{lo}] M2-like macrophages and inflammatory [CD206^{lo}Ly6C^{hi}] M1-like macrophages. Images depict representative animals, graph depicts the ratio between both subsets for all mice. All bar graphs B-E: data are expressed as median with individual data points. Statistical analyses B-E: Mann-Whitney U test. Statistical analysis F: Kruskall-Wallis test; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$.

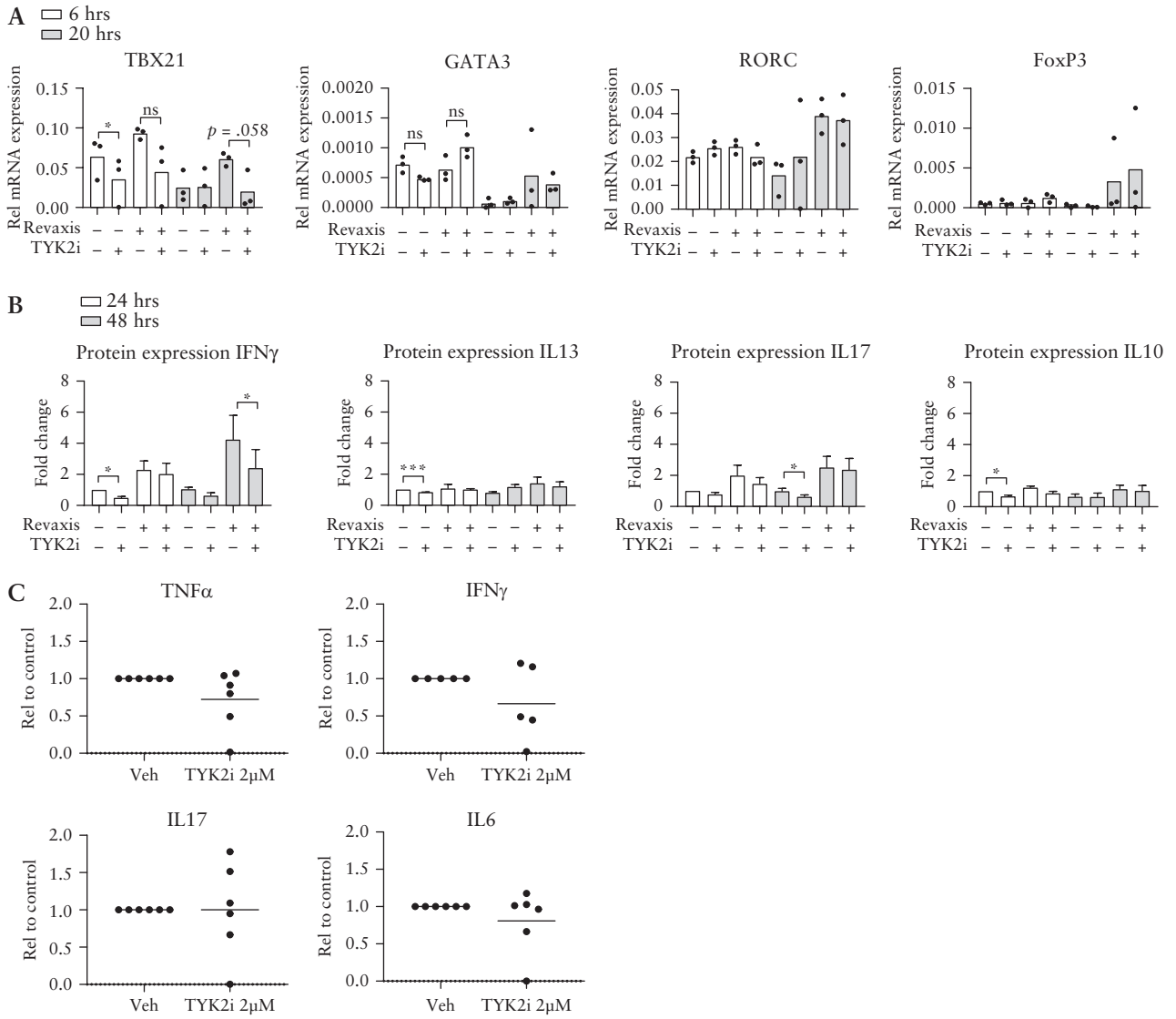

Figure 5. TYK2 inhibition inhibits the differentiation of human Th1 cells. A] Relative mRNA expression normalised to vehicle of T helper [Th] transcription factors Th1 [TBX21], Th2 [GATA3], Th17 [RORC], and T_{reg} [FoxP3], in human lymphocytes incubated with vehicle [DMSO] or the TYK2i [2000 nM] for 24 h co-cultured with dendritic cells [DCs] primed with LPS/Revaxis for 6 or 20 h. B] Protein expression normalised to vehicle of interferon [IFNγ] [Th1], IL17 [Th17], IL13 [Th2], and IL10 [T_{reg}] excreted by human lymphocytes incubated with vehicle or TYK2i and co-cultured with LPS/Revaxis-primed DCs for 24 or 48 h. Data are expressed as mean with standard error of the mean [SEM], each bar consists of three individual donors. C] Patient biopsy material [three UC donors, three CD donors] was incubated with TYK2i [2000 nM] or vehicle [veh; DMSO] for 24 , and protein production of TNFα, IFNγ, IL17, and IL6 was measured in the supernatant. Data are expressed as mean of three triplicates per donor. Statistical analyses A-C: t test [comparisons: vehicle vs TYK2i-treated T cells]; *$p \leq 0.05$; ***$p \leq 0.001$.

In aggregate, our data rather suggest that blocking TYK2 signalling in T cells ameliorates the course of T cell transfer colitis by diminishing the Th1 response. TYK2 deficiency in T cells, rather than in the non-lymphoid cell compartment, is the major contributor to this altered course of colitis. Our data warrant further investigation of the development of TYK2i molecules in human IBD, from both a clinical and a safety perspective.

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Conflict of Interest

MB, RP, and IR are employees of GSK. GDH has served as adviser for Abbvie, Ablynx, Allergan, Amakem, Amgen, AM Pharma, Avaxia, Biogen, Bristol Meiers Squibb, Boerhinger Ingelheim, Celgene/Receptos, Celltrion, Cosmo, Covidien/Medtronics, Ferring, Dr Falk Pharma, Eli Lilly, Engene, Galapagos, Genentech/Roche, Gilead, GlaxoSmithKline, Immunic, Johnson and Johnson, Lycera, Medimetrics, Millenium/Takeda, Mitsubishi Pharma, Merck Sharp Dome, Mundipharma, Nextbiotics, Novonordisk, Otsuka, Pfizer/Hospira, Prometheus laboratories/Nestle, Protagonist, Robarts Clinical Trials, Salix, Samsung Bioepis, Sandoz, Setpoint, Shire, Teva, Tigenix, Tillotts, Topivert, Versant, and Vifor; received speaker fees from Abbvie, Biogen, Ferring, Johnson and Johnson, Merck Sharp Dome, Mundipharma, Norgine, Pfizer, Samsung Bioepis, Shire, Millenium/Takeda, Tillotts, and Vifor;received

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Author Contributions

Study design and conduct of the study, laboratory analyses, and writing of the manuscript: LDV; study design and supervision, and writing of the manuscript: RP, IRP, BS, MM, GD, MW, WdJ; laboratory analyses: LDV, MG, MB, PK, CV; technical support animal experiments: PVH, OW; histological and endoscopic analyses: MW.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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