HHS Public Access

Author manuscript

Cytokine. Author manuscript; available in PMC 2021 December 01.

Published in final edited form as:

Cytokine. 2021 December ; 148: 155715. doi:10.1016/j.cyto.2021.155715.

Divergent functions of IL-17-family cytokines in DSS colitis: Insights from a naturally-occurring human mutation in IL-17F

Chunsheng Zhou[1], **Dongwen Wu**[1,2], **Chetan Jawale**[1], **Yang Li**[1], **Partha S. Biswas**[1], **Mandy J. McGeachy**[1], **Sarah L. Gaffen**[1,*]

[1]Dept of Medicine, Division of Rheumatology and Clinical Immunology, University of Pittsburgh, Pittsburgh PA, USA

[2]The Xiangya Hospital, Gastrointestinal Department, Central South University, Changsha, Hunan, PR China

Abstract

The IL-17 family is structurally distinct from other cytokine subclasses. IL-17A and IL-17F, the most closely related of this family, form homodimers and an IL-17AF heterodimer. While IL-17A and IL-17F exhibit similar activities in many settings, in others their functions are divergent. To better understand the function of IL-17F *in vivo,* we created mice harboring a mutation in *Il17f* originally described in humans with unexplained chronic mucosal candidiasis (Ser-65-Leu). We evaluated *Il17f*$^{S65L/S65L}$ mice in DSS-colitis, as this is one of the few settings where IL-17A and IL-17F exhibit opposing activities. Specifically, IL-17A is protective of the gut epithelium, revealed when trials of anti-IL-17A biologics in Crohn's disease failed and recapitulated in many mouse models of colitis. In contrast, mice lacking IL-17F are resistant to DSS-colitis, partly attributable to alterations in intestinal microbiota that mobilize Tregs. Here we report that *Il17f*$^{S65L/S65L}$ mice do not phenocopy *Il17f*$^{-/-}$ mice in DSS colitis, but rather exhibited a worsening disease phenotype much like *Il17a*$^{-/-}$ mice. Gut inflammation in *Il17f*$^{S65L/S65L}$ mice potentially correlated with reduced Treg accumulation and lowered intestinal levels of *Clostridium* cluster XIV. Unexpectedly, the protective DSS-colitis phenotype in *Il17f*$^{-/-}$ mice could be reversed upon co-housing with *Il17f*$^{S65L/S65L}$ mice, also correlating with *Clostridium* cluster XIV levels in gut. Thus, the *Il17f*$^{S65L/S65L}$ phenotype resembles an IL-17A deficiency in the setting of DSS colitis.

Introduction

The IL-17 cytokine family is unique among cytokine subclasses, with distinct ligand receptor structures, characteristic modes of signaling and diverse biological activities [1, 2]. IL-17A and IL-17F are the signature cytokines of Th17 cells and are 55% identical at

*Correspondence: Division of Rheumatology and Clinical Immunology, BST S702, 200 Lothrop St., Pittsburgh PA 15261, USA. Phone: 412-383-8903. sarah.gaffen@pitt.edu.

Author Contributions

Conceptualization, SLG; Methodology, SLG, CZ; Investigation, CZ, DW, Writing – original draft, CZ, SLG; Writing – Review & Editing, CZ, SLG, PSB, MJM Visualization, CZ, SLG; Supervision, SLG, PSB, MJM; Funding Acquisition, SLG, PSB, MJM

Conflicts of Interest

SLG has consulted for Eli Lilly. The authors declare no other conflicts of interest.

the amino acid level [3]. IL-17A and IL-17F are produced as homodimers and can also exist as an IL-17AF heterodimer [4, 5]. All three isoforms signal through a receptor composed of IL-17RA and IL-17RC, but interact with this receptor with different binding affinities and exhibit correspondingly different signaling strengths: IL-17AA>IL-17AF>IL-17FF [6]. Recent work suggests that IL-17F may also be able to use alternate receptor configurations [7–9].

IL-17 and IL-17F came into prominence with the recognition of Th17 cells as a distinct effector T cell subset in 2005. The discovery of Th17 cells resolved many existing paradoxes regarding the drivers of autoimmune inflammation, and further suggested that IL-17A and/or IL-17F might be viable targets for autoimmune therapy [10, 11]. In 2015 the first antibodies neutralizing IL-17A were approved for moderate-to-severe psoriasis [12], and strategies targeting IL-17A or IL-17RA have proven to be remarkably effective in this setting [13–15].

The success of first-generation anti-IL-17A biologics prompted consideration of IL-17A blockade for treatment of other autoimmune conditions, such as Crohn's Disease (CD). Unexpectedly, in clinical trials of CD, disease was worsened in patients receiving anti-IL-17A or anti-IL-17RA neutralizing antibodies. In fact, trials of brodalumab (anti-IL-17RA) were halted before study completion [16, 17]. Consistent with this, inflammatory bowel disease (IBD) has emerged as a side effect of anti-IL-17A biologic therapy in treatment of psoriasis [13]. These findings are congruent with the majority of studies in mouse models of colitis. *Il17ra*$^{-/-}$, *Act1*$^{-/-}$, *Il17a*$^{-/-}$ or mice treated with IL-17A neutralizing antibodies show exacerbated experimental colitis, shown in the dextran sulfate sodium (DSS)- or TNBS-induced models of epithelial damage (Table 1) [18–23] as well as in other colitis models [24–26]. Resulting mechanistic studies have revealed a dominant role of IL-17A in promoting intestinal epithelial repair (reviewed in [27]).

The role of IL-17F in colitis, on the other hand, appears to be distinct from IL-17A in IBD. The absence of IL-17F in DSS colitis led to an improved outcome (Table 1), suggesting that IL-17F is normally pathogenic in this setting [28, 29]. This resistant phenotype was attributed in part to regulation of enteric microbiota by IL-17F (for example, *Clostridium* cluster XIVa), causing increased levels of intestinal resident Tregs that can mediate tissue protection [28].

Further insights into IL-17F came from an unusual family with chronic mucosal candidiasis (CMC), who harbored a heterozygous serine-to leucine mutation in the *IL17F* gene at position 65 after the leader sequence (IL-17F.S65L) [30]. In mice and humans, IL-17RA or IL-17RC deficiency leads to profound susceptibility to candidiasis, but this was thought to be mainly due to IL-17A [31–33]. Replacement of IL-17F Ser-65 with a non-polar leucine residue impairs the binding of the IL-17F to IL-17RA. Thus, this mutation limits the binding capacity of IL-17F to its receptor but does not interfere the dimerization of IL-17F, either with itself or with IL-17A, and appears to have dominant negative activity [34]. Recently, we reported the creation of mice carrying the IL-17F.S65L mutation [30] to probe functions of IL-17F in candidiasis and other conditions. Whereas *Il17f*$^{-/-}$ mice are resistant to oral candidiasis, *Il17f*$^{S65L/S65L}$ mice showed susceptibility to OPC, which was similar to *Il17a*$^{-/-}$

mice [35]. Collectively these data suggest that IL-17F impacts IL-17A function, which can be revealed with the IL-17F.S65L mutation.

The human family carrying the IL-17F/S65L mutation is lost to followup, and no other individuals carrying this mutation have been reported. Therefore, the *Il17f*$^{S65L/S65L}$ mutant mice provide a tool with which to explore the roles of IL-17F (and by inference IL-17AF) in the context of additional diseases. As noted, DSS colitis is one of the few known settings where IL-17A and IL-17F exhibit dichotomous outcomes [28, 29] (Table 1), so we used this system to test the hypothesis that the IL-17F.S65L mutation is more like IL-17A than like IL-17F. In this report, we first reproduce prior findings that IL-17A is protective in DSS colitis, whereas IL-17F is pathogenic. Strikingly, *Il17f*$^{S65L/S65L}$ mice showed an exacerbated colitis phenotype that is similar to *Il17a*$^{-/-}$ mice (i.e., exacerbated disease) and different from *Il17f*$^{-/-}$ mice. Interestingly, our studies also revealed a dominant role for microbiota in determining the role of IL-17F in colitis, because co-housing *Il17f*$^{S65L/S65L}$ with *Il17f*$^{-/-}$ mice reversed the protective phenotype of the latter animals. Together, these findings are consistent with the capacity of the IL-17F.S65L mutation to impact IL-17A (and/or IL-17AF) *in vivo*, demonstrating that this mouse model can shed light on these enigmatic cytokines.

Methods

Mice

Il17f$^{S65L/S65L}$ mice are available at the MMRRC [35]. *Il17ra*$^{-/-}$ mice were a kind gift from Amgen. *Il17a*$^{-/-}$ *and Il17f*$^{-/-}$ mice were generously provided by Dr. Y. Iwakura, University of Tokyo. WT mice were from The Jackson Laboratory, Taconic Farms, or generated from breeding colonies. All mice were on the C57BL/6 background. Age matched mice of both sexes (6-10 weeks) were used for experiments. Animal use protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

DSS Colitis

2% or 2.5% of DSS (MP bio) in drinking water was given for 7 days. A disease activity index score was a combined assessment of daily weight loss, stool consistency, and bleeding, defined as: Weight loss: 0 (no loss), 1 (1-5%), 2 (5-10%), 3 (10-20%), and 4 (>20%); Stool consistency: 0 (normal), 2 (loose stool), and 4 (diarrhea); Bleeding: 0 (no blood), 2 (visual pellet bleeding), and 4 (gross bleeding, blood around anus). Colon lengths were measured at sacrifice.

Flow Cytometry, FITC Dextran analysis

Epithelial cells were isolated from colon tissue by incubation in RPMI with 3% FBS, 5mM EDTA (Invitrogen) and 1 mM DTT (Invitrogen). Colonic tissue was incubated in a digestion cocktail containing RPMI with 0.5mg/ml DNase and 20μg/ml liberase (Roche). To assess gut epithelial integrity, FITC dextran (Sigma-Aldrich) was administered (40 mg/100 g body weight in 200 uL) via oral gavage. After 4 h, mice blood was collected by cardiac puncture into an EDTA-coated tube. Plasma was separated by cetrifugation. Concentrations of FITC

in plasma were measured by BioTek Plate Reader with an excitation of 485 nm and an emission wavelength of 528 nm (20 nm band width).

qPCR

RNA was extracted using RNeasy kits (Qiagen). cDNA was generated using a Superscript III First Strand Synthesis System (Invitrogen). Relative quantification of mRNA expression was determined by real-time PCR with SYBR green (Quanta BioSciences) normalized to *Gapdh* on the Applied Biosystems 7500 platform. Primers were from QuantiTect (Qiagen).

Enteric C. XIVa determination

Fecal samples and bacterial DNA were isolated with the QIAamp Fast DNA Stool Mini Kit (Qiagen). Quantification of *Clostridium* XIVa DNA was peformed by real-time PCR with Perfecta SYBR Green FastMix ROX (Quanta BioSciences) normalized to the amount of total bacteria (16S) on a 7300 Real-Time PCR System (Applied Biosystems).

Statistics

Data were analyzed on Prism (Graphpad) using ANOVA or Student's t test.

Results

IL-17A and IL-17F play opposing roles in DSS colitis

IL-17A and IL-17F are reported to play opposing roles in DSS colitis (Table 1), but there are some conflicting observations in the literature [36] and outcome can be influenced by enteric microbiota [28]. Therefore, we first evaluated the respective functions of IL-17A versus IL-17F in DSS colitis in our animal facility. To that end, *Il17a*$^{-/-}$ and *Il17f*$^{-/-}$ mice or WT controls (C57BL/6) were given 2% DSS in drinking water for 7 days, replaced by normal water. Weight was assessed daily, and colon length was measured upon sacrifice. Consistent with prior studies, mice lacking IL-17A (*Il17a*$^{-/-}$) or IL-17RA (*Il17ra*$^{-/-}$) lost considerably more weight than WT control mice, with most requiring sacrifice by day 8 for humane reasons (Fig 1A). Commensurate with this, colon lengths of *Il17a*$^{-/-}$ mice were shorter than WT mice (Fig 1B). Even though IL-17F mediates signal transduction through the same receptor complex as IL-17A (namely, IL-17RA and IL-17RC) [2], *Il17f*$^{-/-}$ mice were resistant to colitis, losing less weight than WT mice (Fig 1A). *Il17f*$^{-/-}$ colons were significantly longer than WT, also indicative of less disease (Fig 1B). Therefore, these data importantly verify in our facilities that IL-17F is indeed pathogenic in DSS, in contrast with IL-17A.

Il17f$^{S65L/S65L}$ mice show elevated susceptibility to DSS colitis

A family harboring an IL-17F.S65L mutation were identified with CMC, including oropharyngeal candidiasis (OPC) [30]. Since the sequence surrounding the Serine-65 residue in IL-17F is evolutionarily conserved between mice and humans, we created *Il17f*$^{S65L/S65L}$ knockin mice, which showed an OPC susceptibility profile similar of *Il17a*$^{-/-}$ mice rather than *Il17f*$^{-/-}$ mice [35]. Since DSS colitis is one of the few known conditions where IL-17A and IL-17F exhibit opposing phenotypes, we interrogated the impact of this mutation in

the context of intestinal inflammation. Accordingly, *Il17f*$^{S65L/S65L}$ mice were administered 2% DSS in drinking water and scored for disease severity based on weight loss, stool consistency and rectal bleeding. Upon sacrifice, colon length was measured. DSS treatment caused *Il17f*$^{S65L/S65L}$ mice to lose more weight (Fig 2A), exhibit higher disease scores (Fig 2B), and have shorter colon lengths than WT mice (Fig 2C). To test epithelial integrity in the gut epithelium, *Il17f*$^{S65L/S65L}$, WT mice or untreated controls were given oral FITC-Dextran after 7 days of DSS treatment and plasma was assessed for fluorescence. *Il17f*$^{S65L/S65L}$ mice showed the same plasma FITC-Dextran concentrations as WT mice (Fig 2D), indicating that intestinal barrier function is similar to WT mice in the context of DSS colitis.

Colonic Tregs in Il17f$^{S65L/S65L}$ mice

Il17f$^{-/-}$ mice were previously reported to have elevated proportions of colonic Tregs compared to WT mice, and ablation of Tregs in *Il17f*$^{-/-}$ mice reversed the impact of IL-17F deficiency [28]. To understand how the IL-17F.S65L mutation promotes severity of DSS colitis, we evaluated colonic CD4^{+}Foxp3^{+} cells in *Il17f*$^{S65L/S65L}$ mice at day 9 post-DSS treatment. As shown, proportions of CD4^{+}Foxp3^{+} Treg cells were decreased in DSS-treated *Il17f*$^{S65L/S65L}$ compared to WT mice (Fig 3). However, Treg levels in *Il17f*$^{-/-}$ mice were less than WT, despite their resistance to colitis, perhaps due to the modest protection in colitis observed in this setting.

The intestinal microbiota has been linked to Treg levels and protection from colitis in *Il17f*$^{-/-}$ mice, particularly *Clostridium* cluster XIVa [28]. Therefore, we postulated that cohousing *Il17f*$^{-/-}$ with *Il17f*$^{S65L/S65L}$ mice might enhance *C.* XIVa levels and confer protection against DSS-induced inflammation. Surprisingly, cohousing *Il17f*$^{S65L/S65L}$ mice with *Il17f*$^{-/-}$ mice for two weeks did not alter the severity of DSS colitis in *Il17f*$^{S65L/S65L}$ mice. Rather, the cohoused *Il17f*$^{-/-}$ mice lost resistance to colitis, showing weight loss similar to *Il17f*$^{S65L/S65L}$ mice (Fig 4A). Consistent with this, the colons of cohoused *Il17f*$^{-/-}$ mice were substantially shorter than the non-cohoused controls, indicative of more severe disease (Fig 4B). Fecal *C.* XIVa levels from *Il17f*$^{S65L/S65L}$ mice were also lower than in WT mice. However, the levels of *C.* XIVa in *Il17f*$^{-/-}$ and WT mice were comparable (Fig 4C), suggesting that *Il17f*$^{S65L/S65L}$ mice for some reason do not favor colonization Clostridium cluster XIVa or that the microbiota of *Il17f*$^{-/-}$ mice influences susceptibility to DSS colitis in a manner not attributable solely to *Clostridium* cluster XIVa.

Discussion

Although IL-17F is often viewed synonymously with IL-17A, these cytokines are functionally distinct, especially in the context of mucosal immunity [2, 37, 38]. The goal of this study was to shed some light on the roles of IL-17F in intestinal autoimmunity, taking advantage of the S65L mutation originally described in humans [30]. The IL-17AF heterodimer was shown to exist over ten years ago [4–6], yet remains enigmatic and is challenging to evaluate experimentally due to a paucity of reagents that can selectively block this molecule. The IL-17F.S65L mutation in humans was suggested to function in a dominant-negative manner, blocking both IL-17F and IL-17AF in fibroblasts [30], raising

the possibility that a mouse homologue might be a valuable tool to infer activities of IL-17F and IL-17AF *in vivo.*

Consistent with findings in humans, in OPC the *Il17f*$^{S65L/S65L}$ knockin mice show a phenotype more similar to *Il17a*$^{-/-}$ mice (i.e., susceptible to disease) rather than to *Il17f*$^{-/-}$ mice (resistant) [35] (Table 1). This might suggest that in mice, as in humans, this mutation acts in a dominant-negative manner. However, arguing against a dominant-negative function was the observation that the susceptibility to OPC was only observed in mice homozygous for this mutation, though it is possible that the murine OPC system is simply not sensitive enough to detect this. Similarly, in the DSS-colitis model described here, *Il17f*$^{S65L/S65L}$ knockin mice show a disease phenotype much more similar to *Il17a*$^{-/-}$ than to *Il17f*$^{-/-}$ mice. There was no obvious phenotype in mice heterozygous for the IL-17F.S65L mutation (data not shown), also indicating that IL-17F.S65L is more likely to be a loss of function mutation than a dominant-negative mutation. However, the mechanism underlying how a loss of function mutation in IL-17F could negatively impact the function of IL-17A is unclear. One hypothesis is that the primary biological activities of IL-17A occur through an IL-17AF heterodimer rather than an IL-17A homodimer, but proving this is challenging due to the lack of appropriate tools to explore the functions of IL-17AF heterodimer *in vivo.* Along these lines, the IL-17F.S65L mutation in homodimeric form retains the capacity to signal at high concentrations [35]; for technical reasons we were unable to create a sufficiently pure IL-17A/IL-17F.S65L mutant heterodimer to test the hypothesis that this would be hypofunctional.

The development of biologic agents targeting IL-17A was a major advance in the toolkit of therapies for autoimmunity. The initial studies of anti-IL-17A drugs were remarkably successful in psoriasis, exceeding expectations [14, 39, 40]. However, trials of brodalumab were halted due to worsening of disease [16, 17]. Studies in animals have been somewhat conflicting. Early work reported that *Il17a*$^{-/-}$ mice appear to have an increased severity after DSS treatment [36], but more sophisticated studies of the pathway in mice DSS colitis hinted that this might be case, since mice lacking Act1, IL-17RA, IL-17RC or IL-17A all showed worse disease than WT counterparts (Table 1). Mechanistically, IL-17A was found to promote intestinal repair through several mechanisms, including induction of repair enzymes as well as cooperative signaling with FGF that mediates intestinal repair and regulation of tight junction proteins [19, 21, 25]. Thus, while chemical-induced colitis models are admittedly not an ideal reflection of the true pathogenesis of human IBD, findings using this system have been quite predictive with respect to involvement of the IL-17 pathway.

While there are many settings where *Il17a*$^{-/-}$ mice exhibit more severe alterations in disease compared to *Il17f*$^{-/-}$ mice [2, 37], DSS colitis is one of the few where IL-17A and IL-17F mediate opposing biologic activities. The oral cavity and the intestinal mucosa are similar in that they can both be colonized with *C. albicans,* but unlike the mouth, invasive disease in the gastric mucosa is extremely rare. Therefore, principles that dictate mucosal immunity in the intestine cannot necessarily be extrapolated to the mouth, or vice versa. The implications for therapy are important, as current anti-IL-17A biologics target IL-17A or both IL-17A and IL-17F either through blocking the common receptor (brodalumab) or

bispecific targeting IL-17A and IL-17F (e.g., bimekizumab [41, 42]. Accordingly, blockade of IL-17F might be a viable strategy for intervention in IBD. Nonetheless, a caveat comes from the observation that the disease-protective phenotype in IL-17F-deficient mice could be reversed by co-housing, and thus the composition of the intestinal microbiota can influence disease susceptibility and the impact of cytokine impairment. As described above, the phenotype of *Il17a*$^{-/-}$ mice in DSS colitis model is variable, potentially because of different microbiota across facilities (Table 1). This conflict could also be explained by the different microbiota across facilities. Indeed, Clostridium cluster XIVa did not correlate with disease severity. This was unexpected based on prior reports and suggests that microbiome-mediated protection varies by environment [28, 29]. Clearly more studies are needed to determine the constituents of the microbiota responsible for these observations.

While IL-17F is best understood to signal through a homodimer of IL-17RA and IL-17RC, alternative configurations of the receptor are proposed to exist, and it is unclear which receptor complexes are used by an IL-17F ligand with the S65L mutation. A crystal structure of an IL-17F-IL-17RC homodimer suggests that IL-17F may bind in an IL-17RA-independent manner [7]. It is unclear whether this form of the receptor is signaling-competent, but we previously reported that a forced homodimer of mouse IL-17RC (achieved using a chimera of the IL-17RA extracellular domain fused to the IL-17RC cytoplasmic tail) expressed in *Il17ra*$^{-/-}$ cells failed to mediate signal transduction [43]. Whether this also applies to human IL-17RC is unknown. Along these lines, recent studies have suggested alternative receptor complexes may be used by IL-17A as well [44, 45], so the possibilities for how these cytokines interact with target cells are continuing to expand.

In summary, IL-17F continues to be a puzzling cytokine. The data presented here confirm multiple reports that IL-17F, unlike IL-17A, is destructive in the context of DSS colitis, but that its effects are influenced strongly by the intestinal microbiome, likely through actions on Tregs. Clearly more work needs to be done in this regard to understand the intricate relationships between IL-17F and related cytokines in the context of autoimmune inflammation.

Acknowledgments

NIH supported SLG (AI128991, AI147383), PSB (AI142354, AI159058), MJM (AI148356). We thank BM Coleman for valuable technical assistance. *Il17ra*$^{-/-}$ mice were a kind gift from Amgen. *Il17a*$^{-/-}$ and *Il17f*$^{-/-}$ mice were a generous gift from Dr. Y. Iwakura, University of Tokyo. We thank Dr. Alison Kohan for helpful suggestions.

Abbreviations

CD	Crohn’s Disease
CMC	chronic mucosal candidiasis
DSS	dextran sulfate sodium
IBD	inflammatory bowel disease
OPC	oropharyngeal candidiasis

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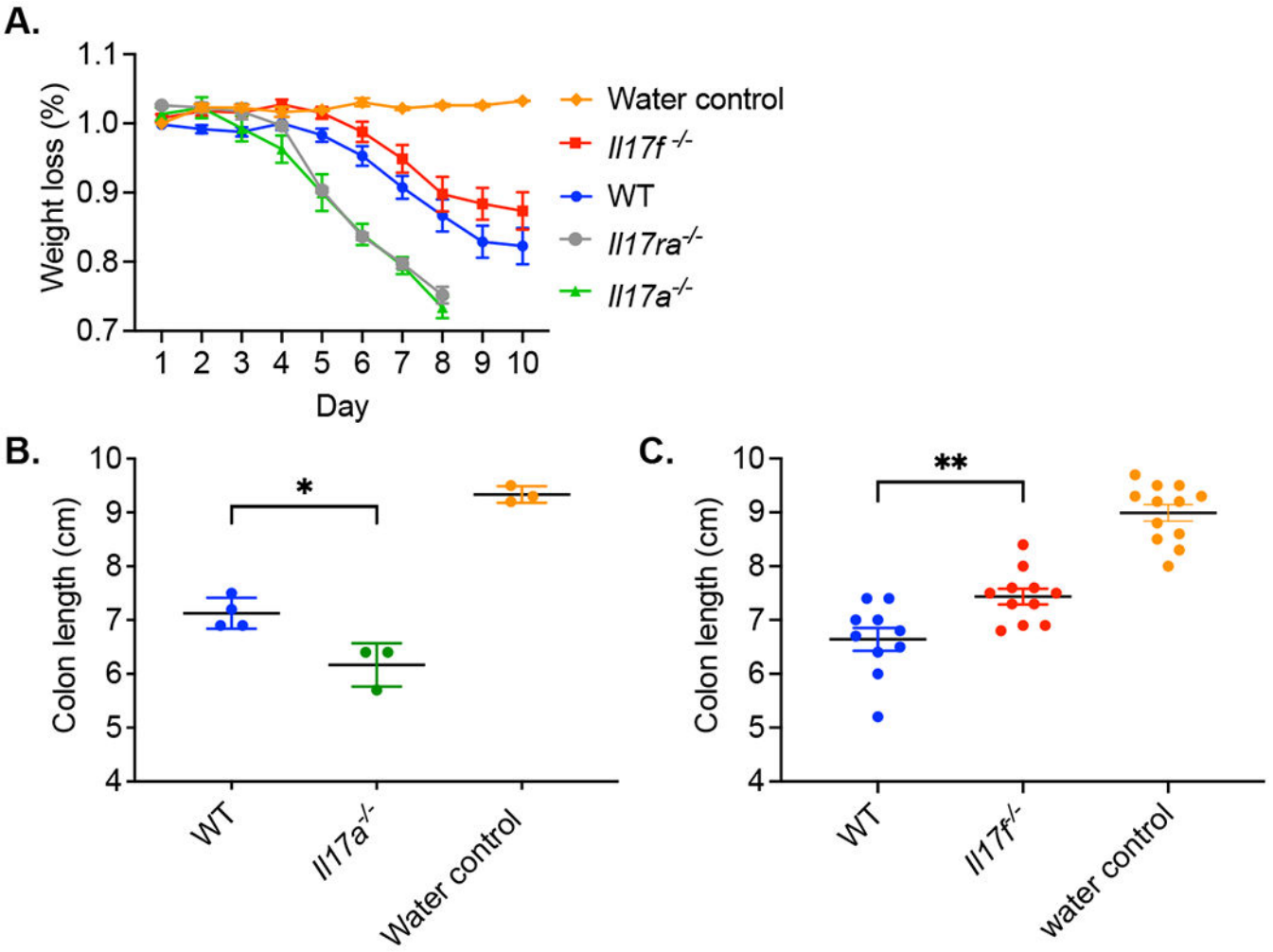

Figure 1. IL-17A and IL-17F show opposing phenotypes in DSS-induced colitis.

The indicated mice were treated with 2% DSS-containing water for 7 days and replaced with normal drinking water for 3 days. (A) Body weight was monitored daily and the percentage relative to starting weight was shown for each time point. Data are compiled from 5 independent experiments. (B) Colon lengths of *IL17a*$^{-/-}$ mice were determined at time of sacrifice (day 8). (C) Colon lengths of *Il17f*$^{-/-}$ mice were measured at sacrifice (day 10). Data were compiled from 3 experiments. Each dot represents one mouse. Graph shows mean ± SEM. Data were analyzed by student t test. *P < 0.05, **P < 0.01.

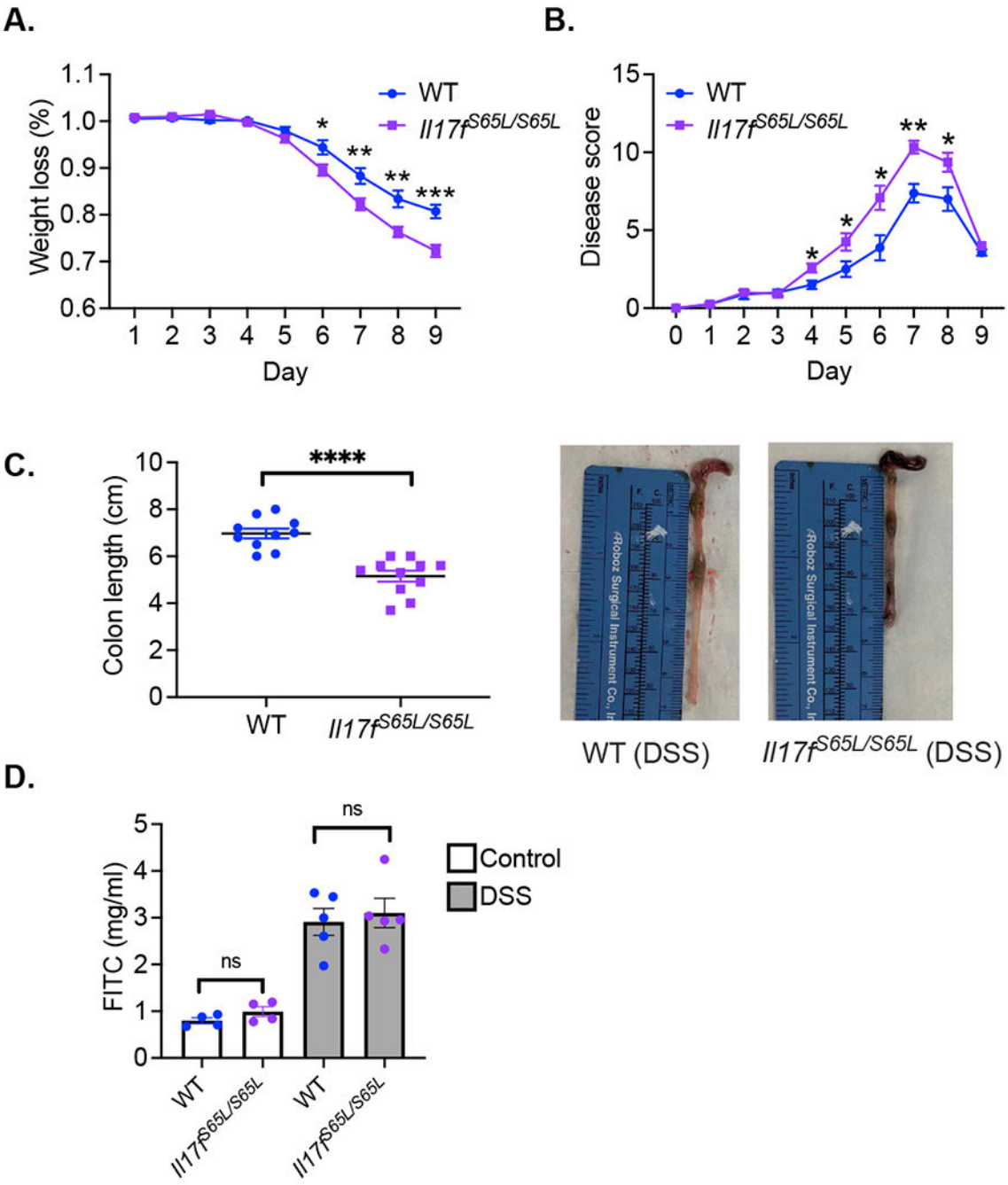

Figure 2. *Il17f*$^{S65L/S65L}$ mice are susceptible to DSS-induced colitis.

The indicated mice were treated with 2% DSS-containing water for 7 days, which was replaced with normal water for another 2 days. (A) Body weight was monitored daily and percentage of starting weight is shown for each time point. (B) Disease scores were assessed daily based on weight loss, anal bleeding and stool consistency. (C) Colon lengths were measured at day 9. Each dot represents one mouse. Graph shows mean ± SEM. Data compiled from 3 independent experiments. Right: Representative colon images. (D) The indicated mice were gavaged orally with FITC-dextran at day 7 post DSS treatment. 4 h

later plasma FITC-dextran concentrations were determined by spectrophometry. Filled bars indicate DSS-treated mice, Empty bars indicate water-treated mice. Graph shows mean ± SEM. Data compiled from 2 independent experiments and analyzed by ANOVA and student t test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.

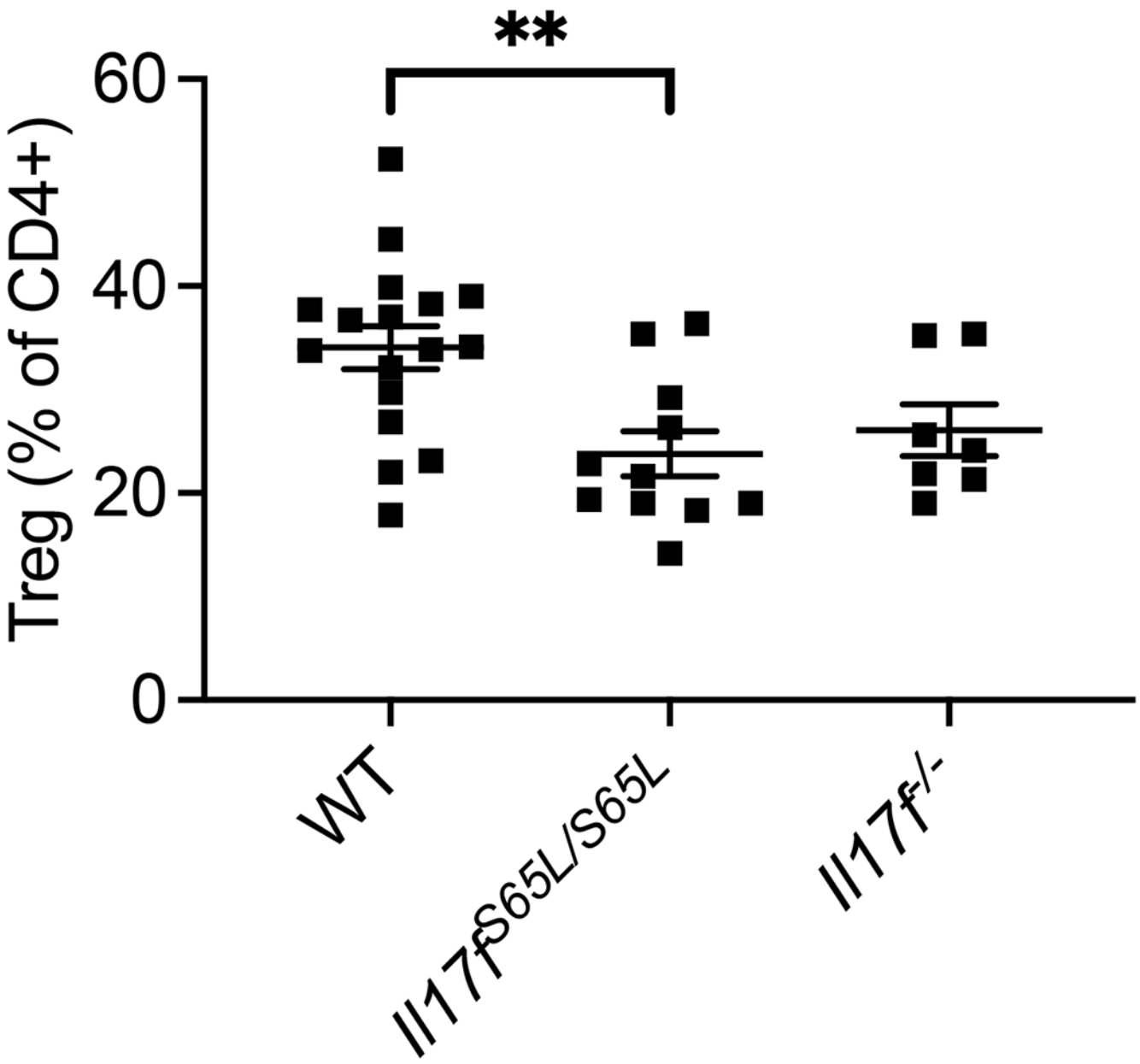

Figure 3. *Il17f*$^{S65L/S65L}$ mice show reduced Foxp3^{+} Treg cells in colon

The indicated mice were given 2% DSS in drinking water for 7 days replaced with normal drinking water for 2 days. Single cell suspensions from colon were analyzed by flow cytometry. Cells were gated on the CD45^{+} and CD4^{+} live populations and stained for Foxp3. Compiled results from 2 independent experiments are shown. Graph shows mean ± SEM. Data were analyzed by Student's t-test. ***P < 0.001

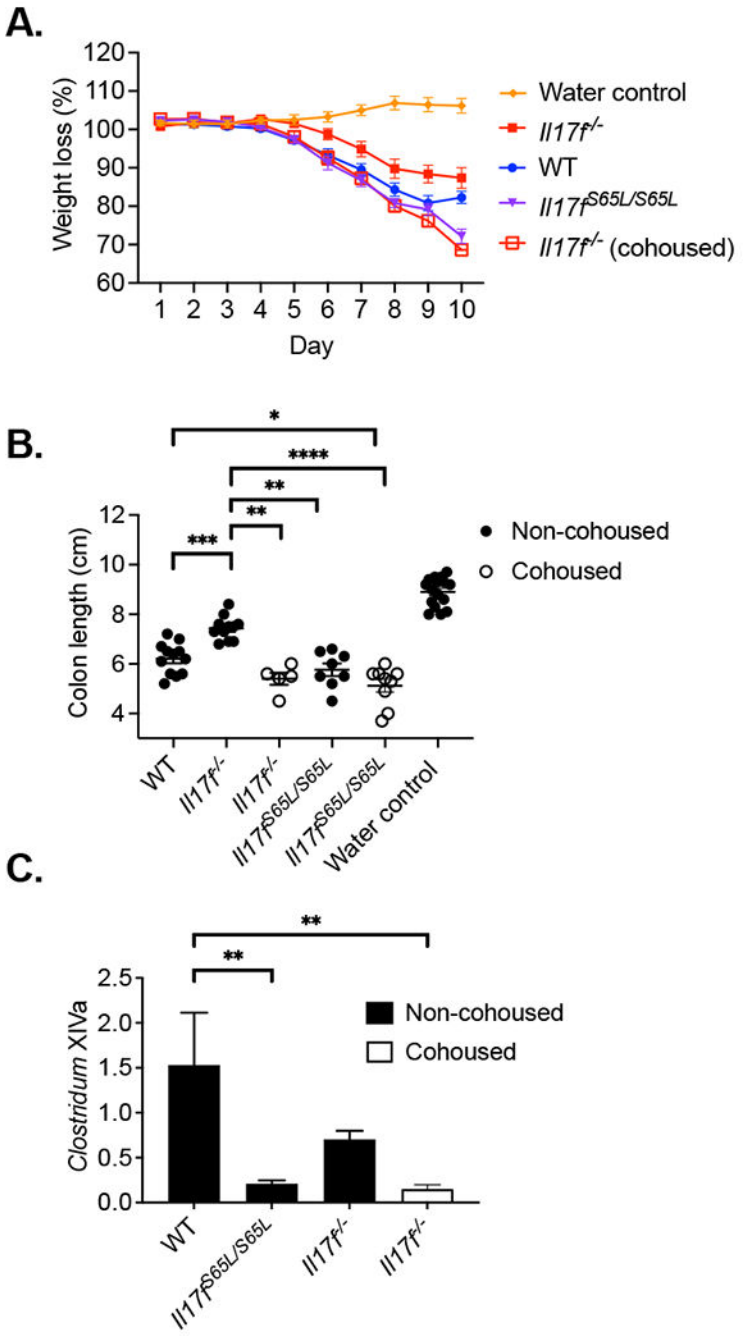

Figure 4. The effects of IL-17F are influenced by the microbiome.

Il17f$^{S65L/S65L}$ mice were cohoused with *Il17f*$^{-/-}$ mice for two weeks, followed by a week of 2% DSS treatment. (A) Body weight was monitored daily and the percentage relative to starting weight is shown. (B) Colon lengths of indicated mice were determined at day 10 post DSS treatment. Each symbol represents an individual mouse. Graph shows mean ± SEM. Data compiled from 3 individual experiments. (C) Feces from the indicated mice collected prior to DSS treatment were assessed for *C. XIVa* levels by PCR. Graph shows

mean ± SEM. Data compiled from 2 independent experiments. **P < 0.01 by ANOVA and Student's t-test or Mann-Whitney analysis

Table 1.

The IL-17/IL-17 receptor signaling axis in chemical-induced colitis

LIGAND/RECEPTOR/MOLECULE	FUNCTION IN COLITIS	SELECTED REFERENCES
IL-17A	Protective	[18] [19] [20] [21]
IL-17A	Pathogenic	[36]
IL-17F	Pathogenic	[28] [29]
IL-17RA	Protective	[46] [25]
IL-17RC	Protective	[23]
ACT1	Protective	[22]