Low dose CP-690,550 (tofacitinib), a pan-JAK inhibitor, accelerates the onset of experimental autoimmune encephalomyelitis by potentiating Th17 differentiation

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**ABSTRACT**

Th17 cells, which have been implicated in autoimmune diseases, require STAT3 signaling activated by IL-6 or IL-23 for their development. Other Th1 and Th2 cytokines such as IL-2, IFN-γ and IL-4 strongly suppress Th17 development. Recently, CP-690,550 (tofacitinib), originally developed as a JAK3 inhibitor, has been shown to be effective in phase III clinical trials of rheumatoid arthritis and collagen-induced arthritis (CIA) models, but the precise mechanism of the effect, especially with respect to Th17 cells, is poorly understood. To our surprise, a low dose CP-690,550 was found to accelerate the onset of experimental autoimmune encephalomyelitis (EAE) at a concentration that suppressed CIA. At an early stage after immunization, more IL-17 production was observed in 15 mg/kg body weight CP-690,550-treated mice than in untreated mice. In vitro, CP-690,550 inhibited both Th1 and Th2 development, while promoting Th17 differentiation at 10–50 nM concentrations. Enhancement of Th17 by CP-690,550 is probably due to suppression of IL-2 signaling, because anti-IL-2 antibodies cancel the Th17-promoting effect of CP-690,550. CP-690,550 selectively inhibited IFN-α-induced STAT1, IL-4-induced STAT6 and IL-2-induced STAT5 at 3–30 nM, while suppression of IL-6-induced STAT3 phosphorylation required a concentration greater than 100 nM. In HEK293T cells, CP-690,550 less effectively suppressed JAK1-mediated STAT3 phosphorylation compared with JAK3. These results suggest that CP-690,550 has a different effects among JAKs and STATs, thereby affecting helper T cell differentiation, and murine autoimmune disease models.

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1. Introduction

Cytokines play essential roles in the development, differentiation, and function of myeloid and lymphoid cells [1]. Many of them, including interleukins (ILs), interferons (IFNs), and hematopoietic growth factors, activate the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway. In this pathway, cytokine binding results in receptor oligomerization, which initiates the activation of JAK kinases (JAK1, JAK2, JAK3, and Tyk2). The activated JAKs phosphorylate the receptor cytoplasmic domains, which creates docking sites for src-homology-2 (SH2)-containing signaling proteins. STAT members are major substrates of JAKs.

The JAK/STAT pathway plays essential roles in immune disorders, including inflammatory diseases, allergies, and auto-immune diseases. This is because cytokines are pivotal in activating innate and acquired immune cells, and the effector cytokines induce various responses in non-immune cells. In particular, helper T (Th) cells are crucial not only in adaptive immune responses but also in various types of immune diseases. Immune pathogenesis that results from dysregulated Th1 responses to self or commensal floral antigens promotes tissue destruction and chronic inflammation, whereas dysregulated Th2 responses can cause allergy and asthma. Th17 cells secrete a distinctive set of immunoregulatory cytokines, including IL-17A, IL-17F, IL-22, and IL-21, and have been shown to play essential roles in autoimmune models, such as experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (CIA). For Th1 development, the IFN-γ/STAT1 and IL-12/STAT4 pathways are essential, for induction of IFN-γ and T-bet expression. Th2 development requires the IL-4/STAT6 pathway. Similarly, the IL-6/STAT3 and the IL-2/STAT5 pathways have been shown to be essential for Th17 and regulatory T cells (Treg) development, respectively [1].
Despite the numerous treatment options that are available for autoimmune and inflammatory diseases, there remains a need for an effective and safe immunosuppressive agent. CP-690,550, a small compound identified as a specific JAK3 inhibitor that affects other JAKs to a much smaller degree, was originally developed as an immunosuppressive agent for organ transplantation [2]. However, CP-690,550 has recently been shown to inhibit JAK1, JAK2 and Tyk2 as well [3]. CP-690,550 is now clinical trial for RA patients [4]. Using CIA models, we previously showed that CP-690,550 mostly inhibits IL-6 and RANKL expression in synovial fibroblasts.

Fig. 1. Effects of CP-690,550 on collagen-induced arthritis (CIA) and experimental autoimmune encephalomyelitis (EAE). (A) 6-week-old DBA/1 male mice were given an initial injection of type 2 collagen on day 0, and arthritis was induced with a second injection on day 21. Vehicle (n = 3) or CP690,550 (15 mg/kg/day, n = 5) was administered intraperitoneally every other day from day 21 to day 29. *p < 0.05. (B) Cytokine serum levels in sera of CIA-induced mice treated with vehicle or CP-690,550 were assessed by ELISA (IL-6 at day 30 and TNF-α at day 40). (C) EAE disease course in vehicle-treated and CP690,550-treated mice (n = 6 mice per group). Vehicle or CP-690,550 (15 mg/kg/day) was administered intraperitoneally every other day from day 0 to day 8. *p < 0.05. (D) Cytometric data for IL-17A and IFN-γ production by CD4+ T cells in central nerve system from EAE mice treated with or without CP-690,550 on day 20 after EAE induction. (E) Quantification of infiltrated CD4+ cells in central nerve system and result in D. *p < 0.05. **p < 0.01.
However, the effect of CP-690,550 in vivo and in vitro remained to be investigated, because Th cell differentiation was not affected in these CIA models, despite a reduced proliferation of T cells [5].

We previously reported that Pyridone 6 (P6), a pan-JAK inhibitor developed by Merck Research Laboratories [6], efficiently ameliorated Th2-type allergic diseases but enhanced Th17 development [7,8]. We proposed that imbalances in JAK inhibitors tending toward JAK or STAT specificity may differentially affect Th development. In the current study, we discovered that CP-690,550 significantly enhanced Th17 development, both in vivo and in vitro, when present in a certain range of concentration, and low-dose CP-690,550 promoted the onset of EAE. We found, in vivo, that CP-690,550 did not suppress STAT3 as effectively as it suppressed STAT1, STAT5 and STAT6, probably due to reduced JAK1 suppression activity. We propose that this small imbalance among JAK inhibitors for JAK selectivity in vivo resulted in the observed promotion of Th17.

2. Materials and methods

2.1. Mice

C57BL/6J mice and DBA/1 J mice were purchased from Nihon Jikken Doubutsu (Tokyo, Japan). Animals were maintained under specific pathogen-free conditions in animal facilities certified by the Animal Care Committee of the Keio University School of Medicine. Animal protocols were approved by the same Animal Care Committee.

2.2. Naïve T cell preparation and differentiation

Naïve CD4+CD25−CD62LhiCD44lo T cells were isolated as previously described [9]. Naïve T cell were cultured at 37 °C (5% CO2) in RPMI-1640 (Invitrogen Life Technologies). The medium was supplemented with 10% FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 50 µM 2-mercaptoethanol. The cells were stimulated with 3 µg/ml of plate-bound anti-CD3 (clone 145-2C11) and 0.5 µg/ml of soluble anti-CD28 (eBioscience). For Th1 cell differentiation, the cells were treated with 10 ng/ml mIL-12p70 (R&D Systems) and 5 µg/ml anti-IL-4 (11B11). For Th2 cell differentiation, the cells were treated with 10 ng/ml mIL-4 (PeproTech) and 5 µg/ml anti-IFN-γ (R4-6A2). For Th17 cell differentiation, the cells were treated with 1 ng/ml human TGF-β (R&D Systems), 20 ng/ml human IL-6 (R&D Systems). In Fig. 2A D and E, 5 µg/ml anti-IFN-γ and 5 µg/ml anti-IL-4 were added in Th17 condition. In Fig. 3B, 50 µg/ml anti-Rat IgG2b (eBioscience) or 10 µg/ml anti-IFN-γ plus 10 µg/ml anti-IL-4 plus 30 µg/ml anti-IL-2 (eBioscience) was added in Th17 condition.

Fig. 2. CP-690,550 suppressed Th1 and Th2, but promoted Th17 differentiation in vitro. (A) Naïve CD4+ T cells were cultured in the presence of indicated concentrations of CP-690,550 with 1 µg/ml anti-TCR mAb for 36 h. Proliferation was assessed by [3H] thymidine incorporation during the final 16 h. (B) Cytokine profiles determined by intracellular staining. For intracellular staining, naïve CD4+ T cells were activated under Th1 (B), Th2 (C), or Th17 (D, E) differentiation conditions, cultured in the presence of indicated concentrations of CP-690,550 (see Section 2). T cells were recovered on day 4, restimulated with PMA and ionomycin for 5 h with brefeldin A, and analyzed by FACS. The quadrant percentiles of cells staining positively for the indicated cytokines are shown. One representative data set from three independent experiments is shown.
2.3. Flow cytometric analysis

For intracellular cytokine staining, cells were stimulated for 5 h in complete medium with phorbol 12-myristate 13-acetate (50 ng/ml) and ionomycin (500 ng/ml; both from Sigma–Aldrich) in the presence of brefeldin A (eBioscience). Surface staining was then performed in the presence ofFc-blocking Abs (2.4G2), followed by intracellular staining for cytokines using a fixation and permeabilization kit (eBioscience) according to the manufacturer’s instructions, as previously described [10]. All Abs were obtained from eBioscience. Data were acquired through a BD FACS Canto II and were analyzed with FlowJo software (Tree Star, Ashland, OR, USA).

2.4. Reagent

CP-690,550 was purchased from Selleck Chemicals (Houston, TX, USA). The expression vectors for JAK1, JAK3, and myc-tagged STAT3 are described in previous reports [11,12].

2.5. Experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis (CIA) induction

For the induction of EAE, mice were immunized s.c. on day 0 with 100 µg/mouse myelin oligodendrocyte glycoprotein (MOG) peptide (aa 35–55) (MEVGWYRSPFSRVVHLYRNGK) (Bex), emulsified in CFA (supplemented with 5 mg/ml Mycobacterium tuberculosis), and injected i.p. on days 0 and 2 with 500 ng/mouse of pertussis toxin (Calbiochem) as described previously [13]. CP-690,550 (15 mg/kg) (Selleck Chemicals) was injected i.p. every other day from day 0 to day 8. The following clinical scoring system was used: 0, no disease; 1, tail limpness; 2, hind limb weakness; 3, hind limb paralysis; 4, forelimb weakness; 5, quadriplegia; and 6, death. The scoring was done every 24 h until day 21. The CIA model was performed as previously described [5]. CP-690,550 (15 mg/kg) (Selleck Chemicals) was injected i.p. every other day from day 21 to day 29. Clinical symptoms of arthritis were evaluated visually for each limb and graded on a scale of 0–4: 0, no erythema or swelling; 0.5, swelling of one or more digits; 1, erythema and mild swelling; 2, mild erythema and mild swelling involving the entire paw; 3, erythema and moderate swelling involving the entire paw; and 4, erythema and severe swelling involving the entire paw. The clinical score for each mouse was the sum of the scores for all four limbs (maximum score, 16).

2.6. Proliferation assay

 Cultures were performed at 37 °C (5% CO2) for 52 h and pulsed with 1 µCi of [3H]thymidine (1 µCi/well; PerkinElmer) during the final 16 h of incubation before harvesting. [3H]Thymidine incorporation was determined using a microplate scintillation counter (PerkinElmer), as previously described [14].

2.7. Western blot analysis

Phosphorylation was analyzed by SDS–PAGE and Western blotting as described previously [15], with the following Abs: anti-phospho-STAT1 (Tyr701; Cell Signaling Technology), anti-phospho-STAT3 (Tyr705; Cell Signaling Technology), anti-phospho-STAT6 (Tyr641; Cell Signaling Technology), anti-phospho-Smad2 (Ser465/467; Cell Signaling Technology), anti-STAT1 (E23; Santa Cruz Biotechnology), anti-STAT3 (C20; Santa Cruz Biotechnology), anti-STAT6 (S20; Santa Cruz Biotechnology), anti-cMyc (sc40; Santa Cruz Biotechnology) and anti-Smad2/3 (BD Biosciences Pharminogen). Blots were visualized with the appropriate HRP-conjugated secondary Abs using Chemi-Lumi One L Immunoblot detection reagents (Nacalai Tesque) or Immobilon Western Chemiluminescent HRP Substrate (Millipore). Band intensities were measured using Image J software (http://rsbweb.nih.gov).

2.8. Transfection

HEK293T cells were seeded onto 10 cm dish plates and transfected using polyethylenimine (PEI, MW 25 kDa [Polysciences, Inc.]) in a 3:1 ratio to the amount of DNA. After 12 h, the cells were replated and treated with CP690,550 for 12 h. The cells were then harvested and Western blot analysis was performed.

Fig. 3. CP-690,550 inhibits STAT1, STAT5 but not Smad2 or STAT3 phosphorylation. (A) Naïve CD4+ T cells were stimulated with 20 ng/ml hIL-6 and 1 ng/ml hTGF-β1 for 30 min after being treated with CP-690,550 at indicated concentrations for 60 min. Cells were then lysed and blotted using the indicated Abs. (B) Cytokine profiles determined by intracellular staining. For intracellular staining, naïve CD4+ T cells were activated with anti-TCR mAb, 20 ng/ml hIL-6 and 1 ng/ml hTGF-β1 with indicated antibody (anti-Rat IgG2b (50 µg/ml); top panels, anti-IFN-γ (10 µg/ml), anti-IL-4 (10 µg/ml) and anti-IL-2 (30 µg/ml); bottom panels) in the presence or absence of CP-690,550 (30 nM).
2.9. Enzyme-linked immune-adsorbent assay

ELISA assays for IL-6 and TNF-α were undertaken following the manufacturer’s instructions (eBioscience). The optical densities at 450 nm were read using a Labsystems Multiscan MS (Analytical Instruments).

2.10. Statistical analysis

All data were analyzed by Student’s t test. A p-value of <0.05 was considered to be significant. All error bars shown in this article represent standard deviations.

3. Results

3.1. CP-690,550 accelerated the onset of EAE

It has been previously shown that CP-690,550 suppressed a type2 CIA model [16]. We confirmed that low dose (15 mg/kg body weight) CP-690,550 efficiently inhibits the CIA model (Fig. 1). In this condition, serum IL-6 and TNF-α levels were reduced by CP-690,550 treatment (Fig. 1B). We applied the same CP-690,550 dose for the EAE model (Fig. 1C–E) and found that CP-690,550 treatment accelerated the onset of EAE (Fig. 1C). Even 1.5 mg/kg body weight CP-690,550 administration exacerbated EAE (data not shown). CP-690,550-treated mice showed greater CD4+ infiltration by the number of cells expressing IL-17 (Fig. 1D and E). The fraction of IFN-γ+ cells remained the same between control and CP-690,550-treated mice. We noticed a much higher number of IL-17+IFN-γ+ Th cells infiltrated into the central nerve system of CP-690,550-treated mice than was observed in control mice. Collectively, these data suggest that CP-690,550 promoted pathogenic Th17 development in vivo and accelerated the onset of EAE.

3.2. CP-690,550 suppressed Th1 and Th2, but promoted Th17 differentiation in vitro

Next, we investigated the effect of CP-690,550 on CD4+ Th cell differentiation. As shown Fig. 2A, CP-690,550 did not affect anti-TCR antibody-induced CD4+ T cell proliferation (≈100 nM), suggesting that CP-690,550 did not affect cell viability or proliferation at a concentration of 100 nM. 50 nM, but not 10 nM, CP-690,550 suppressed IFN-γ production 4 days after Th1 differentiation conditions were established (Fig. 2B), while both 10 nM and

![Fig. 4. CP-690,550 differentially inhibits JAKs and STATs activation. (A and B) Naïve CD4+ T cells were cultured with indicated cytokine for 30 min after being treated with CP-690,550 at indicated concentrations for 60 min; cells were then lysed and blotted with the indicated Abs. See data of Fig. 4 of Ref. [7] for the data of cytokines (++,+) in the absence of drugs. (C) HEK293T cells were transfected with Myc-tagged STAT3 and JAK1 or JAK3. After 12 h, the cells were treated with CP-690,550 at indicated concentrations for 12 h; cells were then lysed and blotted using the indicated Abs. The relative ratio of the band intensity of phosphorylated STAT3 versus that of Myc-tagged STAT3, which normalized by the level of CP (0 nM) intensity, is shown in the right. Leukemia inhibitory factor (LIF) (10 ng/ml) was used for positive control stimulant.](image-url)
That the Th1 and Th2 cytokines IFN-

to Th2 differentiation and, that Th2 is more
sensitive than Th1 to this drug. Similar preferential suppressive ef-

fected on Th2 have been observed with Pyridone 6, another pan-JAK

inhibitor [7]. We then examined the effect of CP-690,550 on Th17

and induced T regulatory (iTreg) cells. As shown in Fig. 2D and E,

CP-690,550 enhanced IL-17 production while suppressing Foxp3 and

IL-10 induction in a dose-dependent manner under Th17 differen-

tiation conditions. These data indicate that <100 nM CP-

690,550 efficiently inhibits Th1, Th2 and iTreg while promoting

Th17, in vitro.

3.3. Low dose CP-690,550 inhibits STAT1, STAT5 but not Smad2 or

STAT3 phosphorylation

To investigate the molecular mechanisms of the effects on Th17

promotion, we examined the activation status of STATs under Th17

differentiation conditions in the presence of various concentrations

of CP-690,550. We found that STAT1 and STAT5 were phosphory-

lated under Th17 differentiation conditions, and that phosphoryla-

tion was suppressed by CP-690,550 in a dose-dependent manner

(Fig. 3A). However, neither Smad2 nor STAT3 phosphorylation

were suppressed at CP-690,550 concentrations up to 100 nM

(Fig. 3A).

STAT1 and STAT5 can be phosphorylated by IFN- and IL-2 from

Th cells under Th17 differentiation conditions. It has been shown

that the Th1 and Th2 cytokines IFN-γ, IL-4 and IL-2, strongly sup-

press Th17 differentiation. Therefore, we examined the effect of

anti-cytokine antibodies during Th17 differentiation in the pres-

ence or absence of CP-690,550. As shown in Fig. 3B, combinations of

anti-IFN-γ, anti-IL-4 and anti-IL-2 antibodies strongly enhanced

Th17 differentiation and almost completely cancelled the enhance-

ment by CP-690,550 (Fig. 3B). These data suggest that CP-690,550

enhanced Th17 differentiation not by promoting TGF-β/IL-6 signal-

ing but by suppressing Th1 and Th2 differentiation and/or Th1/Th2

cytokine production.

3.4. CP-690,550 differentially inhibits activation of JAKs and STATs

Promotion of Th17 by CP-690,550 in vivo could be explained by a

differential effect of this compound on JAKs and STATs. To con-

firm this interpretation, we measured the effect of CP-690,550 on

each STAT phosphorylation by a specific cytokine. Naive CD4+ T

cells isolated from mice were stimulated with various cytokines

in the presence of various concentrations of CP-690,550. As shown in

Fig. 4A, CP-690,550 selectively inhibited IFNγ-induced STAT1, IL-

4-induced STAT6, and IL-2-induced STAT5 at 3–30 nM, while

30 nM CP-690,550 did not suppress IL-6-induced STAT3 phos-

phorylation. A concentration greater than 100 nM was required for the

partial suppression of STAT3 (Fig. 4B).

It has been shown that JAK1 is a major JAK that mediates IL-6

signaling [17], so we compared the suppression efficiency of CP-

690,550 for JAK1 and JAK3 (Fig. 4C). JAK1 or JAK3 was overexpres-

sed together with STAT3 in HEK293T cells, and the levels of STAT3

phosphorylation by transfected JAKs were examined. CP-690,550

showed a strongly suppressive activity for JAK3, while it had a

weaker effect on JAK1-mediated STAT3 phosphorylation (Fig. 4C).

These data suggest that CP-690,550 has a weaker effect on JAK1

in vivo compared with its effect on JAK3, and therefore has a weak-

er inhibitory effect on STAT3, resulting in differential effects for

each Th development.

4. Discussion

We found that CP-690,550, a potent JAK inhibitor, exacerbated

EAE by enhancing Th17 development in vivo. This is an unexpected

effect because CP-690,550 has been shown to be therapeutically

effective for CIA models [16] as well as human RA patients [4].

We previously showed that CP-690,550 inhibits IL-1-mediated

RANKL and IL-6 expression in synovial fibroblasts, and IL-6-mediat-

ed STAT3 phosphorylation at concentrations ranging from 100 to

1000 nM in a CIA model [5]. We found that, in mice, a 15 mg/

kg body weight dose of CP-690,550 strongly suppressed CIA, but

promoted EAE. This suggests that CIA, characterized by synovial

fibroblast hyperplasia and bone destruction, is not strongly depen-

dent on Th17, while EAE is much more dependent on Th17 cells.

In CIA models, we previously showed that CP-690,550 inhibited T cell

proliferation but not differentiation strongly, and that CP-690,550

efficiently inhibited STAT3 activation in synovial fibroblasts [5].

Although it is not clear why Th cells were not particularly affected

by CP-690,550 in the CIA models, CP-690,550 may be concentrated

in joints more efficiently than in secondary lymphoid organs.

We should emphasize that CP-690,550 can actually inhibit IL-6 signal-

ing at >100 nM in fibroblasts as well as in T cells, and promotion of

Th17 is mostly a consequence of the relatively severe suppression

of Th1, Th2 and iTregs, and not due to enhanced STAT3 activation.

CP-690,550 mostly inhibits synovial fibroblasts, therefore seems to

be therapeutic for CIA and RA by suppressing the JAK/STAT3 path-

way in these cells.

In an EAE model, we found clear enhancement of Th17 cell infil-

tration into the central nerve system in CP-690,550-treated mice.

We also showed that another JAK inhibitor, Pyridone 6, also inhibits

Th1 and Th2 but enhances Th17 both in vitro and in vivo [7]. These

results suggest that the effect of JAK inhibitors on Th differentiation

in vivo may be dependent on immunization protocol and/or adju-

vants, given different levels of environmental cytokines. This diffe-

rential skewing effect of JAK inhibitors on Th differentiation may be

beneficial for treating certain immunological diseases. For example,

suppression of Th2 may be the most important therapeutic effect

for allergic diseases. Moreover, the relative enhancement of Th17

by Pyridone 6 was observed to be rather effective for curing inflam-

matory dermatitis in NC/Nga mice, because Th17 cytokines pro-

mote skin barrier repair and antimicrobial peptide expression [7].

Thus, CP-690,550, like Pyridone 6, must be ideal for the treatment

of atopic dermatitis. We propose that not only selective inhibition

of Th development, but also modulation of Th balance or correction

of Th imbalance may be particularly effective. JAK inhibitors with

different sensitivities to particular STATs could still be valuable for

the treatment of different immunological disorders.

We showed that CP-690,550 promoted Th17 differentiation by

suppressing Th1 and Th2 cytokine production, mostly because

CP-690,550 inhibited STAT1, STAT5 and STAT6 more efficiently

than STAT3. The reason for the differential suppressive effect of

CP-690,550 has not yet been clarified. As shown in Fig. 4C, we

found that CP-690,550 has a lower suppressive effect on JAK1 than

on JAK3 in vivo. The reduced sensitivity of JAK1 for CP-690,550 may

explain the weaker suppressive effect of CP-690,550 on STAT3, be-

cause IL-6 and related cytokines mostly utilize JAK1 [17]. However,

apparently, CP-690,550 strongly suppresses JAK1 tyrosine kinase

in vitro. It has been reported that IC50s of CP-690,550 are 1.6 nM

for JAK1, 21.7 nM for JAK2 and 6.45 nM for JAK3 in vitro [3]. Thus,

CP-690,550 may be less effective for JAK1 in vivo compared with

in vitro. There are two possibilities that may explain this behavior.

First, CP-690,550 may not be able to access the entire JAK1 mole-

cule in vivo, because only the tyrosine kinase domain was used

for the kinase assay in vitro. Second, JAK1 parts other than the

kinase domain may have a reduced affinity for CP-690,550. Alter-
natively, JAK1-associated molecules including receptors reduced the affinity of JAK1 to the drug in vivo. Chemical modification of JAK1 inducing phosphorylation may also reduce the affinity for the drug. Further biochemical study is necessary, to define the molecular basis for the observed differential effect of JAK inhibitors on each JAK.

It is believed that Th17 is deeply involved in certain type of autoimmune diseases, including psoriasis and inflammatory bowel disease (IBD). Th17-specific inhibitors such as RORγt inhibitors have been developed, and have been shown to be effective in EAE models [18]. Although the mechanism has not been completely elucidated, halofuginone has been shown to be a Th17-selective inhibitor [19]. Our study suggests that JAK1-specific or STAT3-specific inhibitors could be Th17-selective, to a greater degree than for Th1 and Th2. Development of such inhibitors could lead to novel and effective therapies for autoimmune diseases.

5. Disclosures

The authors have no conflicting financial interests.

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