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BET Bromodomain Inhibition Suppresses HIF-1α-Mediated IL-17 Expression in Peripheral Blood Mononuclear Cells from Patients with Rheumatoid Arthritis

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Abstract: Objectives: The purpose of this study was to explore the potential of the bromodomain and extra-terminal domain (BET) bromodomain to regulate IL-17 expression in peripheral blood from patients with rheumatoid arthritis (RA) and its underlying mechanisms. Methods: The level of IL-17A, TNFα and IFNγ in PBMCs from patients with RA was evaluated by a cytometric bead array. The IL-17A and IFNγ production in the supernatants of splenocytes and the serum level of IL-17A in mice were detected by ELISA. The intracellular cytokines were measured by flow cytometric analysis. The protein expression was measured using western blot. Results: This study show that the presence of JQ1 decreased the product and mRNA expression of IL-17A, but not IFNγ and TNFα, in anti-CD3/anti-CD28-stimulated peripheral blood mononuclear cells (PBMCs) from treatment-naive patients with early RA. The percentages of IL-17A-expressing CD4+ T cells were also reduced by JQ1 in stimulated PBMCs. JQ1 also inhibited the expression of the transcription factor retinoic acid receptor-related orphan receptor-γt (RORγt) and T-bet. Furthermore, JQ1 inhibited hypoxia-inducible factor-1α (HIF-1α) expression, but did not affect activity of mammalian target of rapamycin complex 1 (mTORC1). HIF-1α inhibitor reduced percentage of IL-17A- expressing CD4+ T cells. Conclusions: This study indicated that the epigenetic readers BET bromodomain might contribute to regulating HIF-1α-mediated IL-17 expression in RA. BET bromodomain inhibition might be a novel therapeutic approach for RA.

Keywords: Bromodomain and Extra-Terminal Domain, Th17, Rheumatoid Arthritis, HIF-1α

1. Introduction

Rheumatoid arthritis (RA) is a common chronic autoimmune disease characterized by dysregulated immune responses, chronic synovial inflammation and progressive joint destruction [1]. Increasing evidence suggests that IL-17A-producing T helper 17 (Th17) cells have important roles in the development of autoimmune diseases including RA [2-6]. Elevated proportions of Th17 cells were found in the peripheral blood and synovial tissue from patients with RA [7-9]. The deletion of IL-17A improves the severity of arthritis in a murine collagen-induced arthritis (CIA) model [10-12]. IL-17A also modulates bone destruction of RA by promoting the expression of RANKL, a critical factor for osteoclast differentiation, in osteoblasts and in fibroblasts-like synoviocytes [13,14]. These observations indicate that the modulation of targeting Th17 cell might be a future approach for RA treatment [5].

A number of intracellular signaling cascades and transcriptional factors are involved in differentiation and activation of Th17 cells. Recent studies indicate that mammalian target of rapamycin complex 1 (mTORC1) and hypoxia-inducible factor-1 (HIF-1) play important roles in regulating Th17 differentiation positively [15]. Stimulation of TCR, CD28 and cytokine receptors activates mTORC1 in CD4+ T cells [16,17]. Activated mTORC1 enhances HIF-1α

expression, and then HIF-1α interacts with RORγt and p300 in order to induce optimal transcription at the IL17 gene and other Th17 associated loci [18,19].

Members of the bromodomain and extra-terminal domain (BET) family of bromodomain proteins (BRD2, BRD3, BRD4 and BRDT) contain bromodomain motifs that bind acetylated lysine residues in histones and connect acetylated chromatin and gene transcription [20,21]. Previous reports have demonstrated that the suppression of BET bromodomain inhibits lipopolysaccharide (LPS) induced production of pro-inflammatory cytokines and chemokines in bone marrow derived macrophages [22, 23]. A recent study revealed that BET bromodomains inhibition results in alterations of cytokine production in T cells [24]. The BET family of chromatin adaptors has fundamental and selective roles in the differentiation of Th17 cells as well as activation of previously differentiated TH17 cells, suggesting that BET inhibition might be a therapeutic intervention in autoimmune diseases including RA [25]. However, the molecular mechanisms that BET bromodomain regulates differentiation and activation of Th17 cells in RA remain unclear. Therefore, this work evaluated the role of the BET bromodomains inhibitor JQ1 in regulating IL-17 expression in the peripheral blood cells from treatment-naive patients with early RA and whether JQ1 controlled IL-17 expression through inhibiting mTORC1-HIF-1α signaling pathway.

2. Materials and Methods

2.1. Patients

Nineteen treatment naive patients with early RA (16 women and 3 men, ages 31-70 years) and 12 healthy volunteers (10 women and 2 men, ages 29-68 years) were studied. All of the patients fulfilled the American College of Rheumatology revised criteria for RA [26] and had experienced inflammatory symptoms for less than 1 year. The clinical and laboratory data of treatment naive patients with early RA are shown in Table 1. Five patients were taking nonsteroidal anti-inflammatory drugs. None of the patients had ever taken disease-modifying anti-rheumatic drugs or corticosteroids. The study was approved by the Medical Ethics Review Board of the First Affiliated Hospital, Sun Yat-sen University, China.

Table 1. *Clinical and laboratory data of patients with RA*.*

Morning stiffness	18 (94)
Arthritis of more than 3 joints areas	17 (89)
Arthritis of hand joints	19 (100)
Symmetric arthritis	19 (100)
Serum rheumatoid factor	15 (79)
Serum anti-CCP	16 (84)
Bone erosion	3 (16)
DAS28, mean ±SEM (range)	6.2±1.3 (4.2-8.6)
Swollen joint count, mean ±SEM (range)	13.5 ±1.4 (4-28)
Tender joint count, mean ±SEM (range)	12.4±1.3 (4-26)
Disease duration, mean ±SEM (range) months	4.2±0.5 (2-9)

*Values are the number (%) of patients unless otherwise indicated. Anti-CCP, anti-cyclic citrullinated peptide; DAS28, disease activity score in 28 joints

2.2. Preparation and Culture of Peripheral Blood Mononuclear Cells

Peripheral blood samples were obtained from patients with early RA and from healthy controls. Peripheral blood mononuclear cells (PBMCs) from RA patients were harvested by Ficoll density gradient centrifugation. These cells were resuspended in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% charcoal-treated FBS, 100 units/ml penicillin, 100 μg/ml streptomycin (BioWhittaker, Walkersville, MD), and 50 μmol/L 2-mercaptoethanol (Life Technologies). The cells (200,000/well) were cultured for 72 hours in 96-well plates. Anti-CD3 (300 ng/ml) and anti-CD28 (400 ng/ml) were used to stimulate the cells in the presence or absence of different concentrations (10, 50, and 100 n*M*) of JQ1 (Selleck). JQ1 was dissolved in demethylsulfoxide (DMSO) at an initial concentration of 1 mM and serially diluted to the working concentrations with RPMI-1640.

2.3. Measurement of Cytokine Production

The supernatants from cell culture were stored at -20°C until use. The production of IL-17A, TNFα and IFNγ in PBMCs from patients with RA was evaluated by a cytometric bead array (CBA) (BD Biosciences, San Jose, CA) according to the manufacturer's instructions. The IL-17A and IFNγ production in the supernatants of splenocytes and the serum level of IL-17A in mice were detected by ELISA according to the manufacturer's instructions. Mouse plasma was obtained by the centrifugation of blood in serum separator Microtainer tubes after cardiac puncture.

2.4. Flow Cytometric Analysis

For the intracellular measurement of cytokines, cells were activated for 4 hours with 50 ng/ml phorbol myristate acetate and 500 ng/ml ionomycin in the presence of GolgiStop (BD Biosciences, Sunnyvale, CA) to detect intracellular cytokines. The cells were harvested and stained with cell surface antigen fluorescein isothiocyanate (FITC)–conjugated CD4 mAb (eBiosciences) followed by standard intracellular staining using Fixation Buffer (eBiosciences) and Permeabilization Buffer (eBiosciences). To eliminate dead cell contamination, 7-AAD (Invitrogen) was used to stain dead cells. The samples were examined on Beckman Coulter flow cytometer and analyzed by Kaluza software (Beckman Coulter). The following mAbs were purchased from eBioscience: allophycocyanin (APC)–conjugated IL-17A, APC-780 conjugated IFNγ, APC-conjugated TNFα, and PE-conjugated CD45RO.

2.5. Western Blot Analysis

The protein concentrations were measured by the BCA protein assay (Pierce, Rockford, IL, USA). Equal amounts of protein were solubilized in Laemmli buffer (62.5 mM Tris/HCl pH 6.8, 10% glycerol, 2% SDS, 5% b-mercaptoethanol and 0.00625 % bromophenol blue), boiled

for 5 min, and then separated by SDS–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were probed with primary antibodies diluted to 1:1000 for RORγt and T-bet in TBS-T containing 5% nonfat milk at 4°C overnight. The membranes were incubated with the appropriate secondary antibodies for 1 hour at room temperature. The immunoreactive bands were visualized by an enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech) reaction. Each blot is representative of at least three similar independent experiments. A high-resolution scanner was used to scan the film. Densitometry was performed using an AlphaEaseFc (Fluorchem8900) system, and the ratio of the optical density (OD) of the target protein to the OD of β-actin bands (relative intensity) was calculated.

2.6. Quantitative Real-Time Polymerase Chain Reaction (PCR) Analysis

The total RNA was extracted using a RNeasy Mini kit (Qiagen). The complementary DNA was synthesized with an Omniscript RT kit (Qiagen). Quantitative real-time PCR was performed using a Qiagen SYBR green PCR kit and a 7300 real-time PCR system (ABI). The relative mRNA expression levels were evaluated using ΔCt values and were normalized to GAPDH. The primers for quantitative RT-PCR are summarized in Table 2.

Table 2. *Sequences of primers used in this study.*

IL-17A	forward	CGGCTGGAGAAGATACTGGTG
IL-17A	reverse	AACTGCTTTGGGGAGTGTGG
IFNγ	forward	TCGGTAACTGACTTGAATGTCCA
IFNγ	reverse	TCGCTTCCCTGTTTTAGCTGC
TNFα	forward	CCTCTC TCTAATCAGCCCTCTG
TNFα	reverse	GAGGACCTGGGAGTA GATGAG
RORγt	forward	AGATACCCTCACCTACACCTTG
RORγt	reverse	CCGCTCAGGGCTGTATTCAA
T-bet	forward	GTCCAACAATGTGACCCAGAT
T-bet	reverse	ACCTCAACGATA TGCAGCCG
GAPDH	forward	GCACCGTCAAGGC TGAGAAC
GAPDH	reverse	TGGTGAAGACGCC AGTGGA

2.7. Statistical Analysis

The data are expressed as the mean ± SEM. An analysis of variance with Student-Newman-Keuls test was used to evaluate the differences between experimental groups. The histologic results were analyzed by the Kruskal- Wallis nonparametric test. *P* values less than or equal to 0.05 were considered significant.

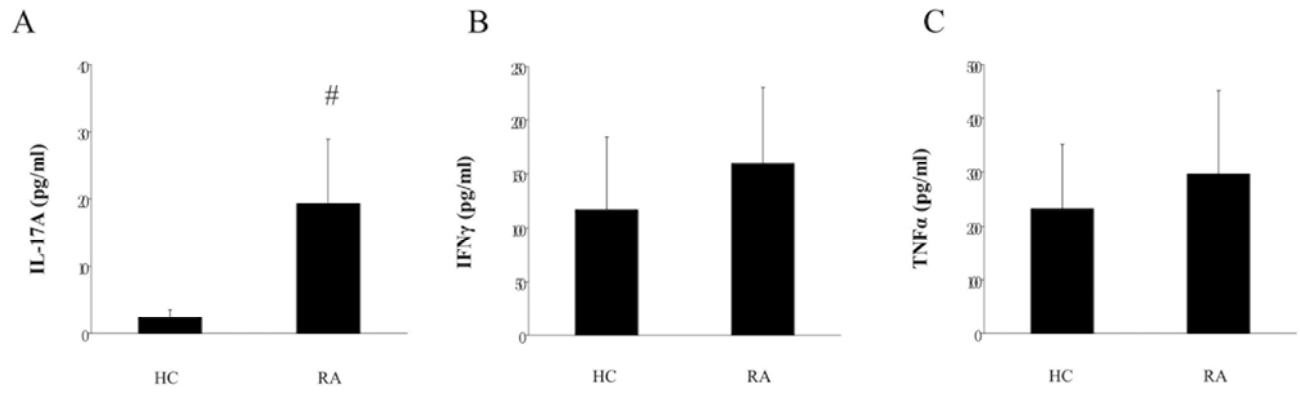
3. Results

3.1. Effects of JQ1 on the Production of IL-17A, TNFα and IFNγ by PBMCs from Treatment- Naive Patients with Early RA

First, the production of IL-17A, TNFα and IFNγ in PBMCs from treatment-naïve patients with early RA and from healthy controls were evaluated. The level of IL-17A in the supernatants of anti-CD3/anti- CD28- stimulated PBMCs from treatment-naïve patients with early RA was increased significantly compared with stimulated PBMCs from healthy controls. However, similar levels of TNFα and IFNγ were shown in the supernatants of anti-CD3/anti-CD28 stimulated PBMCs from patients with early RA and healthy controls (Figure 1A, B and C).

JQ1 is a broad-spectrum BET protein inhibitor that suppresses interactions between multiple BET proteins (Brd2/3/4) and acetylated histones; therefore, JQ1 was used as a specific BET inhibitor in our study. As shown in Figure 1D, JQ1 treatment suppressed the production of IL-17A by PBMCs from RA patients in a dose-dependent manner. However, JQ1 treatment had no effects on the production of the TNFα and IFNγ in anti-CD3/anti- CD28- stimulated PBMCs from these patients (Figures 1E and F).

We also found that JQ1 had no effects on cell death or on the proliferation of anti-CD3/anti-CD28-stimulated PBMCs (data not shown), ruling out possibility of Th17 inhibition as an indirect effect of JQ1 on T cell survival and proliferation. The potential effect of JQ1 on cytokine mRNA expression levels was measured by real time qPCR. As shown in Figures 1G, H and I, JQ1 treatment reduced the mRNA expression of IL-17A by anti-CD3/anti-CD28-stimulated PBMCs from RA patients in a dose-dependent manner. However, JQ1 did not influence the mRNA expression of TNFα and IFNγ in anti-CD3/anti-CD28-stimulated PBMCs from these patients. These results are consistent with our data on cytokine production.



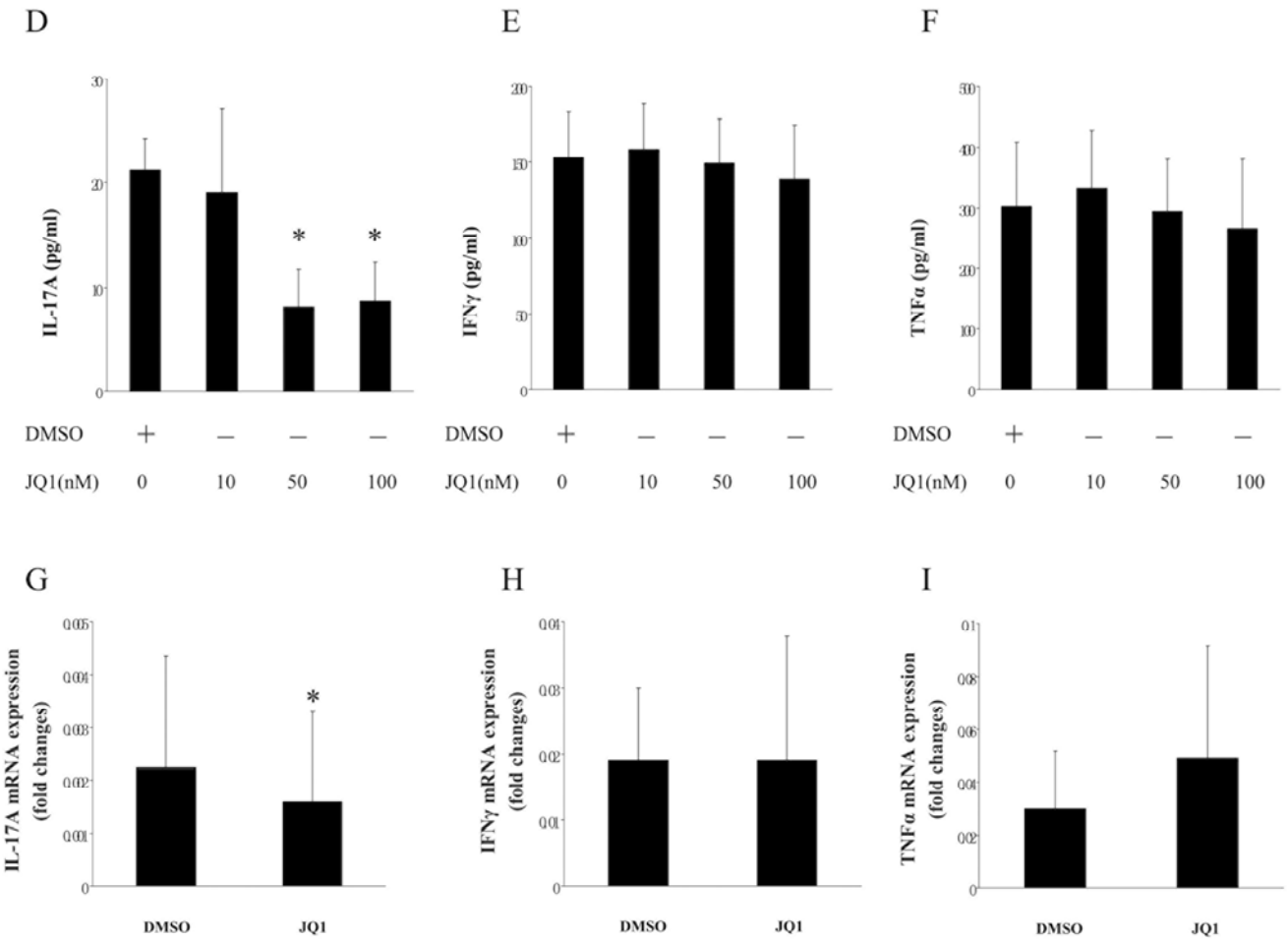


Figure 1. *Effects of JQ1 on the production and gene expression of IL-17A, IFNγ and TNFα by peripheral blood mononuclear cells (PBMCs) from treatment-naive patients with early rheumatoid arthritis (RA).*

A, B and C, production of IL-17A (A), IFNγ (B) and TNFα (C) by cultured PBMCs from healthy controls and from patients with RA. D, E and F, effects of JQ1 on the production of IL-17A (D), TNFα (E) and IFNγ (F) by PBMCs from patients with early RA. PBMCs were cultured for 72 hours with anti-CD3/anti-CD28 in the presence or absence of various concentrations of the BET inhibitor JQ1. The bars represent the mean and SEM cytometric bead array results from cultures of stimulated PBMCs from 19 treatment-naive patients with early RA and from 12 healthy controls. G-I, effects of JQ1 on the mRNA expression of IL-17A (G), IFNγ (H) and TNFα (I) by PBMCs from patients with RA. PBMCs were cultured for 24 hours with anti-CD3/anti-CD28 in the presence or absence of JQ1 (100 nM). Cytokine mRNA expression levels were evaluated by real time PCR. The relative mRNA expression was normalized to GAPDH. *$P < 0.05$ versus DMSO, #$P < 0.05$ versus healthy controls.

3.2. Effects of JQ1 on the Percentages of IL-17A+ and IFNγ+ CD4+ T Cells in PBMCs from Treatment-Naive Patients with Early RA

First, the percentage of IL-17A+ and IFNγ+ CD4+ T cells in treatment-naive patients with early RA and in healthy controls was analyzed by flow cytometry. As shown in Figure 2A, a markedly increased percentage of IL-17A+ and IFNγ+ CD4+ T cells was observed in the PBMCs from treatment-naive patients with early RA compared with healthy controls.

Next, the effects of JQ1 on the percentage of IL-17A+ and IFNγ+ CD4+ T cells from treatment-naive patients with early RA was evaluated. Treatment with JQ1 resulted in a significant reduction of the fraction of IL-17A+ CD4+ T cells in anti-CD3/anti-CD28-stimulated PBMCs from treatment naive patients with early RA. In contrast, the addition of JQ1 did not reduce the IFNγ+ CD4+ T cell fraction (Figures 2B, C and D). In addition, we observed no significant reduction of the fraction of TNFα-expressing CD4+ T cells in anti-CD3/anti- CD28-stimulated PBMCs from treatment-naive patients with early RA by JQ1 treatment (Figure 2E). This result is similar to our previous observations that JQ1 did not inhibit the production of TNFα by PBMCs from RA patients.

PBMCs were cultured for 72 hours with anti-CD3/ anti-CD28 in the presence or absence of various concentrations of JQ1. The intracellular expression levels of cytokines were measured by flow cytometry. A and B, intracellular IL-17A expression in CD4+ T cells (A) and

IFNγ expression in CD4+ T cells (B) in anti-CD3/ anti-CD28-stimulated PBMCs from healthy controls (n =10) and patients with early RA (n = 12), as measured by flow cytometry. C-E, effects of JQ1 on the expression of IL-17A (C), IFNγ (D) and TNFα (E) in CD4+ T cells from treatment-naive patients with RA. The numbers are the percentages of cells within the quadrants. The bars represent the mean and SEM. *P < 0.05 versus DMSO, #P < 0.05 versus healthy controls.

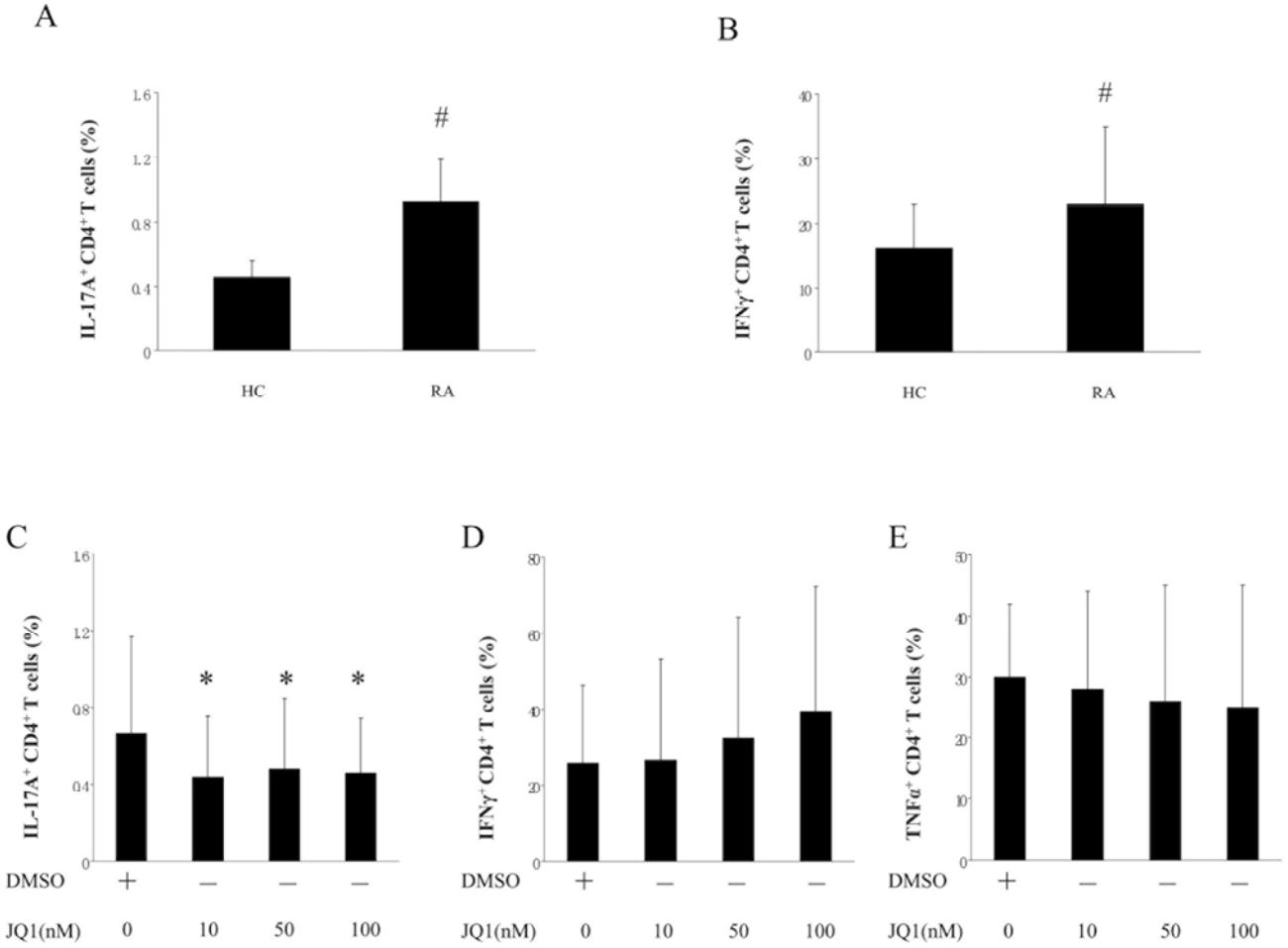


Figure 2. *Effects of JQ1 on percentages of IL-17A+ and IFNγ+ CD4+ T cells in PBMCs from treatment-naive patients with early RA.*

3.3. Effects of JQ1 on the Expression of Transcript Factor RORγt and T-bet in PBMCs from Treatment-Naive Patients with Early RA

As a T cell-specific isoform of retinoic acid receptor-related orphan receptor (ROR), RORγt, encoded by the *RORC* gene, play a critical role in Th17 differentiation; therefore, the effects of JQ1 on the expression of RORγt in PBMCs from patients with early RA was investigated. As shown in Figure 3A, JQ1 treatment significantly suppressed protein expression of RORγt in anti-CD3/anti-CD28–stimulated PBMCs from treatment-naive patients with early RA. However, JQ1 did not influence the protein expression of T-bet, a key transcription factor for the differentiation of Th1 cells. Furthermore, the effects of JQ1 on RORγt and T-bet mRNA expression was evaluated, as determined by real time PCR. Consistent with our expectations, JQ1 treatment decreased the mRNA expression of RORγt, although not T-bet, in anti-CD3/anti-CD28-stimulated PBMCs from treatment-naive patients with early RA (Figure 3B).

A and B, the protein expression of RORγt (A) and T-bet (B) was determined by Western blot analyses. PBMCs were stimulated for 72 hours with anti-CD3/anti-CD28 in the presence or absence of JQ1 (100 nM). The data represent the mean (S.E.M.) of the densitometrical quantification (lower panel). C and D, the mRNA expression of RORγt (C) and T-bet (D) was determined by real time PCR. PBMCs were stimulated for 24 hours with anti-CD3/anti-CD28 in the presence or absence of JQ1 (100 nM). The relative mRNA expression was normalized to GAPDH. *P < 0.05 versus DMSO.

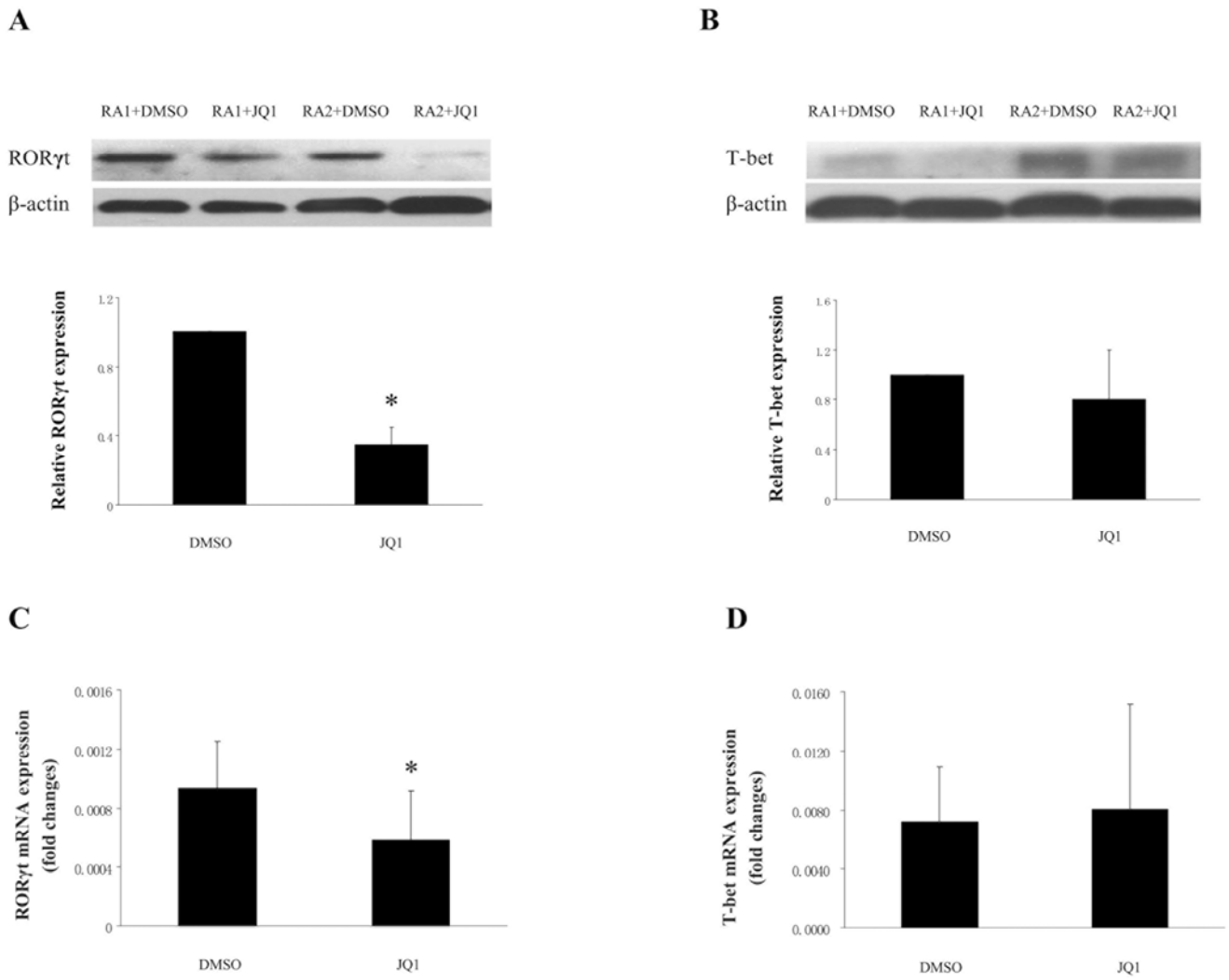


Figure 3. *Effects of JQ1 on the expression of transcript factor RORγt and T-bet in PBMCs from treatment-naive patients with early RA.*

3.4. Effects of JQ1 on mTORC1 and HIF-1α Expression in PBMCs Cells From Treatment-Naive Patients with Early RA

It has been shown the emerging roles of HIF-1α in the differentiation and activation of Th17 cells, therefore, to investigate the molecular mechanisms that BET protein in regulating IL-17 expression, the inhibitory effect of JQ1 on HIF-1α expression in PBMCs from treatment- naive patients with early RA was evaluated firstly. As shown in Figure 4A, JQ1 treatment inhibited the expression of HIF-1α in anti-CD3/ anti-CD28- stimulated PBMCs from treatment-naive patients with early RA. Since mTORC1 activity is required for HIF-1α induction in TH17 cell differentiation [27], the effect of JQ1 on mTORC1 activity was evaluated. However, we demonstrated that mTORC1 activity was not affected by JQ1 in anti-CD3/anti-CD28-treated PBMC (Figure 4B).

PBMCs were stimulated for 72 hours with or without anti-CD3/anti-CD28 in the presence or absence of JQ1 (100 nM). The protein expression of HIF-1α(**A**) and mTORC1 (B) was determined by Western blot analyses. The data represent the mean (S.E.M.) of the densitometrical quantification (lower panel). **P* < 0.05 versus DMSO

3.5. Effects of Inhibitor of HIF-1α on Expression of IL-17 in PBMCs from Treatment-Naive Patients with Early RA

To investigate the role of HIF-1α in IL-17 expression, the PBMCs were pretreated with HIF-1α inhibitor PX-478 2HCl (20uM). As shown in Figure 5, PX-478 2HCl treatment reduced the percentage of IL-17A+ CD4+ T cells in anti-CD3/anti-CD28- stimulated PBMCs from treatment-naive patients with early RA.

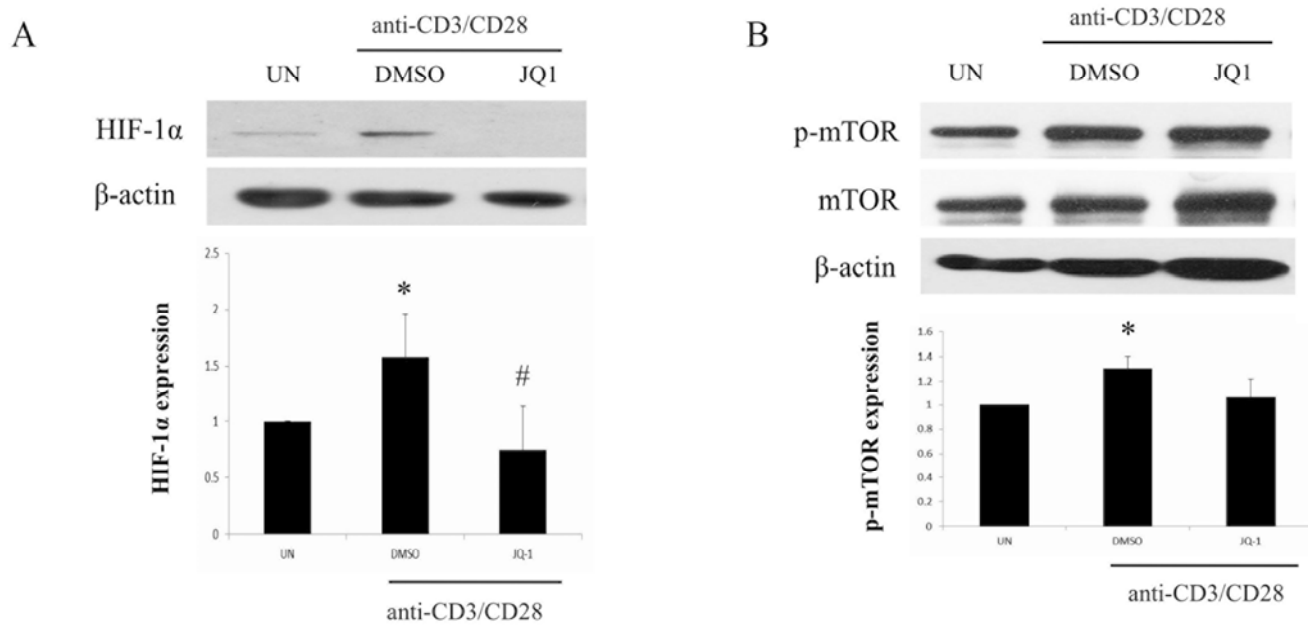


Figure 4. *Effects of JQ1 on the HIF-1αexpression and mTORC1 activity in PBMCs from treatment-naive patients with early RA.*

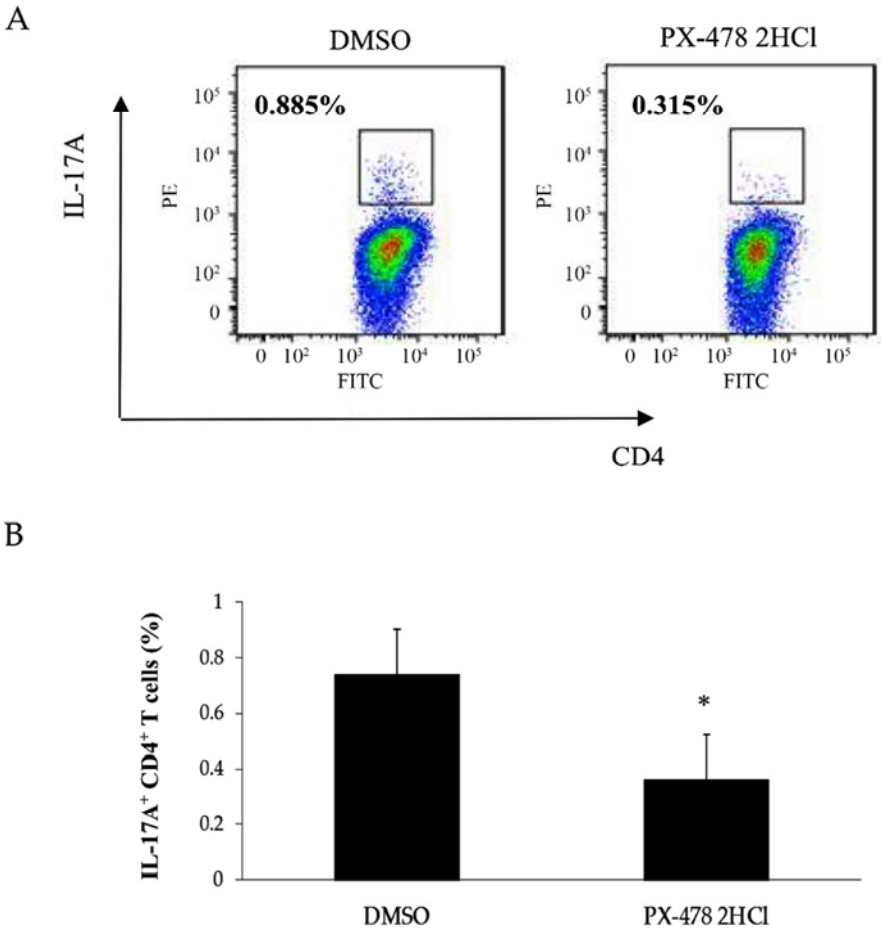


Figure 5. *Effects of HIF-1αinhibitor on percentages of IL-17A+ CD4+ T cells in PBMCs from treatment-naive patients with early RA.*

PBMCs were cultured for 72 hours with anti-CD3/ anti-CD28 in the presence or absence of HIF-1αinhibitor PX-478 2HCl (20uM). The intracellular expression levels of IL-17A were measured by flow cytometry from treatment-naive patients with RA. The numbers are the percentages of IL-17A+ CD4+ T cells within the quadrants (A). The bars represent the mean and SEM from 3 independent experiments (B). *$P < 0.05$ versus DMSO.

4. Discussion

The current work demonstrated that JQ1 treatment decreased the production of IL-17A and the percentage of IL-17A-expressing CD4+ T cells in anti-CD3/anti-CD28-stimulated PBMCs from treatment-naïve patients with early RA. However, JQ1 did not affect the production of IFNγ and TNFα and the percentage of IFNγ- and TNFα- expressing CD4+ T cells in the stimulated PBMCs. This study also observed the inhibitory effect of JQ1 on the expression of RORγt, although not T-bet, in stimulated PBMCs. Furthermore, JQ1 inhibited HIF-1α expression, and inhibition of HIF-1α reduced percentage of IL-17A- expressing CD4+ T cells in this study. These results indicate an important role of BET bromodomain in regulating IL-17 expression in peripheral blood cells of RA.

Th17 cell differentiation and function in humans and animals are associated with susceptibility to RA [28]. IL-17 is produced by activated CD4+ and CD4+CD45RO+ (memory) T cells [29]. The progression of joint destruction and disease activity is associated with the levels of IL-17A and Th17 cells in patients with RA [30,31]. The pathogenic potential of Th17 cells is further confirmed by a reduction in joint inflammation and erosions of patients in a phase I study of LY2439821, a potent humanized anti–IL-17 antibody [32]. These studies imply that Th17 cells might be attractive targets for RA treatment. However, a great challenge in medicine is how to regulate pathogenic Th17 cell activity in RA.

A recent study demonstrated that BET bromodomain has a critical role in regulating Th17 differentiation and the production of Th17- associated cytokines [25]. BET suppression also improves in joint inflammation in mice with CIA [25]. However, it is still unknown whether BET inhibition regulates Th17 cells differentiation and activation in patients with RA. Therefore, we observed the effect of JQ1 on IL-17 expression in PBMCs from treatment-naïve patients with early RA. Our results demonstrated that the BET inhibitor JQ1 reduces the percentage of IL-17A- expressing CD4+ T cells and the production of Th17-associated cytokines IL-17A in anti-CD3/anti-CD28-stimulated PBMCs from patients with early RA. JQ1 also exhibits an inhibitory effect on the expression of RORγt, a key transcription factor for initiating the differentiation program of Th17 cells [33,34]. These findings indicate an important role of BET inhibition in regulating IL-17 expression in memory T cells in RA. Because there is considerable interest in targeting IL-17A or Th17 cells in the treatment of RA and other Th17-mediated diseases [3], BET suppression might be a possible approach. In recent years, accumulating evidence has shown that chromatin "readers", which specifically bind histone posttranslational modifications and provide a framework of an integral element of the transcriptional activation complex [35], might play critical roles in immune and inflammatory responses by regulating chromatin acetylation and inflammatory transcriptional activation via recruitment of coregulatory complexes [36-40]. Therefore, our study suggests important roles of BET bromodomain reader proteins in regulating Th17-mediated inflammation.

Although it has been demonstrated an important role of BET proteins in regulating differentiation and activation of Th17 cells [25], the precise mechanisms are still poorly understood. Under CD3/CD28-stimulated T cells, activated mTORC1 enhances HIF-1α expression and RORγt nuclear translocation [15]. Since a previous study [25] and our present data indicate the inhibitory effect of JQ1 on RORγt expression, this prompts us to evaluate whether BET proteins are involved in RORγt expression through regulating mTORC1-HIF-1α axis. In this study, JQ1 treatment suppressed HIF-1α expression, but did not affect mTORC1 activity in CD3/CD28-stimulated PBMCs from RA patients. HIF-1α inhibitor also reduced the IL-17 expression. These results indicate that the role of BET proteins in controlling HIF-1α expression is not through regulating mTORC1 pathway, however, may be due to a direct effect in HIF-1α gene transcription or be through other alternative upstream signal pathways such as STAT3. Nevertheless, our study firstly provides the evidence that BET bromodomain can modulate IL-17 expression *via* regulating HIF-1α in anti-CD3/CD28- stimulated PBMCs from RA patients. Although it has been indicated that BET proteins drive T cell differentiation into the Th17 subset possibly through regulating the transcriptional state of NF-κB–dependent genes [25], the results reported here might provide a novel mechanism that BET bromodomain controls Th17 cells activation.

Th1-driven autoimmunity is thought to play an important role in the pathogenesis of RA. However, in the present study, JQ1 treatment does not affect the percentage of Th1 cells and the production of the Th1 cytokine IFNγ in anti-CD3/anti-CD28-stimulated PBMCs from treatment-naïve patients with early RA. JQ1 also has no influence on the expression of T-bet, a critical transcription factor for controlling the differentiation of Th1 cells. Our results demonstrated that there was no influence of IFNγ- expressing CD4+ T cells in the spleen and PBMCs in vivo from JQ1-treated mice with CIA. In agreement with our observations, other work previously identified that BET bromodomain inhibition had no effect on the differentiation of human and murine Th1 and Th2 from naive CD4+ T cells [25]. These data indicate that BET bromodomain inhibition might not affect a shift toward Th1 in the development of RA. These results suggest that BET reader proteins might play a selective role in regulating Th17 cells activation in RA.

TNFα has been recognized as a major factor in the pathogenesis of RA. The success of anti-TNFα therapies in the majority of RA patients further reveals the pathogenetic importance of this cytokine [41], however, in the present study, JQ1 treatment did not markedly influence the production of TNFα in anti-CD3/anti-CD28-stimulated PBMCs from RA patients. Moreover, the FACS experiment also demonstrated that the presence of JQ1 did not diminish the percentage of TNFα-positive CD4+ T cells in stimulated PBMCs. In accordance with our data, a previous report

demonstrated that, in anti-CD3/anti- CD28- stimulated differentiated human Th17 cells, JQ1 had no significant influence on the transcript level, on the production of TNFα or on lymphotoxin-α, another TNF superfamily member [25]. In contrast, in LPS-induced murine macrophages, JQ1 markedly inhibits the production of TNFα [23]. These data indicate that the effect of JQ1 on TNFα production appears to be complex and might be associated with the stimulus and with the different cell types in the cell cultures.

5. Conclusion

In summary, the study have demonstrated that BET proteins play important roles in regulating IL-17 expression through HIF-1α in peripheral blood of patients with RA, suggesting that regulating BET-dependent chromatin signaling might provide novel targets for treatment of Th17-associated autoimmune disorders including RA.

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Ethics Approval

Institutional Medical Ethics Review Board of the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China.

Patient Consent

Obtained.

Competing Interests

No conflict of interest has been declared by authors.

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