Bromodomain and extra-terminal domain bromodomain inhibition prevents synovial inflammation via blocking IκB kinase-dependent NF-κB activation in rheumatoid fibroblast-like synoviocytes

Youjun Xiao1,*, Liuqin Liang1,*, Mingcheng Huang1,*, Qian Qiu1, Shan Zeng1, Maohua Shi1, Yaoyao Zou1, Yujin Ye1, Xiuyan Yang1 and Hanshi Xu1

Abstract

Objective. To explore the roles of the bromodomain (Brd) and extra-terminal domain (BET) of chromatin adaptors in regulating synovial inflammation in RA.

Methods. Fibroblast-like synoviocytes (FLSs) were isolated from synovial tissue from RA patients. A specific BET inhibitor, JQ1, and short hairpin RNA (shRNA) for Brd2 or Brd4 were used to evaluate the role of the BET Brd in inflammatory responses. Protein expression was measured by western blot or immunofluorescence staining. Nuclear factor kappa B (NF-κB) gene activity was detected by luciferase assay. The secretion and gene expression of cytokines and MMPs were evaluated by ELISA and real-time PCR, respectively. FLS proliferation was detected by BrdU incorporation.

Results. Four Brd proteins, including Brd2, Brd3, Brd4 and Brdt, were expressed in FLSs from patients with RA and OA; however, the expression of Brd2 and Brd4 was increased in RA compared with that in OA. Treatment with JQ1, Brd2 shRNA or Brd4 shRNA decreased the production of pro-inflammatory cytokines (TNFα, IL-1β, IL-6 and IL-8), MMPs expression (MMP-1, MMP-3 and MMP-13) and proliferation by RA FLSs. BET inhibition downregulated TNFα-induced NF-κB-dependent transcription and expression of the NF-κB target genes. JQ1 suppressed the phosphorylation of IκBα and IκBβ, and nuclear translocation of p65. Intraperitoneal injection of JQ1 in mice with collagen-induced arthritis reduced synovial inflammation, joint destruction and serum levels of the anti-CII antibodies TNFα and IL-6.

Conclusion. This study implicates BET Brds as important regulators of IκB kinase/NF-κB-mediated synovial inflammation of RA and identifies BET proteins as novel therapeutic targets in inflammatory arthritis.

Key words: rheumatoid arthritis, bromodomain and extra-terminal domain, NF-κB, IκB kinase, fibroblast-like synoviocytes

Rheumatology key messages

- Bromodomain and extra-terminal proteins contribute to fibroblast-like synoviocytes-mediated synovial inflammation of RA.
- Bromodomain and extra-terminal inhibition suppresses NF-κB activation through blocking IκB kinase signal in RA fibroblast-like synoviocytes.
- Bromodomain and extra-terminal inhibition may be a new therapeutic approach for RA.

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Introduction

RA is a common chronic inflammatory disease characterized by dysregulated immune responses, chronic synovial inflammation and progressive joint destruction. Proinflammatory cytokines and MMPs play important roles in the initiation and development of synovial inflammation and joint destruction of RA [1, 2].

As an important transcription factor, nuclear factor kappa B (NF-κB) modulates the expression of a host of cellular genes in immune and inflammatory responses [3, 4]. NF-κB is highly activated in the synovium of murine CIA and of patients with RA [5, 6] and plays a key role in regulating the production of synovial inflammation, hyperplasia and joint destruction [7–10]. Thus, targeting NF-κB-mediated synovial inflammation is promising for the development of novel therapies for RA [11]. The mechanisms of NF-κB activation are complex. The canonical pathway involves multiple steps, including the activation of IκB kinase (IKK), the phosphorylation, ubiquitination and degradation of IκBα, and a series of p65 (RelA) subunit post-translational modifications such as acetylation, phosphorylation, and methylation [12].

Members of the bromodomain (Brd) and extra-terminal (BET) family of Brd proteins (Brd2, Brd3, Brd4 and Brdt) contain Brd motifs that bind acetylated lysine residues in histones, connecting acetylated chromatin and gene transcription [13]. As chromatin regulators, BET proteins could recruit transcriptional co-activators such as positive transcription elongation factor b to promote gene transcription in inflammatory conditions [14]. Recent studies have indicated that the BET Brd inhibitor suppresses the lipopolysaccharide (LPS)-induced expression of pro-inflammatory cytokines and chemokines in bone marrow-derived macrophages [15, 16]. Brd4 can promote the transcriptional activation of NF-κB and the expression of a series of NF-κB–mediated inflammatory genes [17]. However, it is unknown whether BET Brd proteins participate in regulating synovial inflammation in RA.

In this work, we assessed the effects of BET Brd inhibition on the NF-κB activation in synovial inflammation of RA, and demonstrated that BET Brds influence early cytoplasmic signalling in the NF-κB pathway through IKK activity in RA fibroblast-like synoviocytes (FLSs). Our study suggests that BET Brd inhibition might possess therapeutic potential for RA.

Materials and methods

Reagents and antibodies

TNF-α was obtained from R&D Systems (R&D Systems, Minneapolis, MN, USA). DMEM/Ham’s F12 (DMEM/F12), fetal bovine serum, antibiotics, trypsin–EDTA, PBS and other reagents for cell culture were purchased from Invitrogen (Carlsbad, CA, USA). JQ1 was purchased from Calbiochem (La Jolla, CA, USA). Collagenase, LPS (Salmonella typhi) and β-actin antibody were purchased from Sigma (St Louis, MO, USA). The p65, IKKα, phospho-IKK, IκBα and phospho-IκBα antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The Brd2, Brd3, Brd4 and Brdt antibodies were obtained from Abcam (Cambridge, MA, USA).

Patients

Synovial tissue samples were obtained from 15 patients with active RA (13 women and 2 men, ages: 45–62 years) and 8 patients with OA (7 women and 1 man, ages: 52–61 years) who were undergoing synovectomy or joint replacement. RA was diagnosed according to the 1987 revised criteria of the ACR [18]. The demographics of RA patients are shown in Table 1. The study was approved by the Medical Ethical Committee of the First Affiliated Hospital at Sun Yat-sen University and was conducted according to the recommendations of the Declaration of Helsinki. All patients provided informed consent for participation in the study.

Preparation and culture of FLSs

The synovial tissue from patients was cut into small pieces and digested with collagenase I in DMEM/F12 medium to isolate the synoviocytes. The synoviocytes were grown in a DMEM/F12 medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified incubator at 37°C under 5% CO2. At confluence, the cells were trypsinized. The cells were used from passage three to five, during which time they are a homogeneous population of cells (<1% CD11b positive, <1% phagocytic and <1% FcgRII and FcgRII receptor positive) and characterized by vimentin and cadherin-11, a FLSs-specific marker [19]. Cell viability was measured using an 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay, which was performed as previously described [20].

Construction of short hairpin RNA-expression lentivirus and infection

The lentivirus-based short hairpin RNA (shRNA) vectors were constructed as described previously [21]. The sequences of these shRNA oligonucleotides are listed in supplementary Table S1, available at Rheumatology online. The shRNA-expression lentiviral vectors were generated by cloning gene-specific or scramble shRNA sequences into pLKO.3G vectors, and lentiviruses were produced through the co-transfection of HEK 293T cells with expression vectors and helper plasmids, pCMV-dR8.2-vprX and pCMV-VS-VSVG, using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. Lentiviral particles were harvested from cell supernatants 48 h and 72 h after transfection. Then, purification was performed using ultracentrifugation, and the titre of lentiviruses was determined. For infection, RA FLSs were treated with lentiviruses and polybrene (10 μg/ml, Santa Cruz) for 12 h at 37°C. Subsequently, the virus-containing medium was replaced with fresh medium. After 3 days of infection, the FLSs were used to perform the experiments. The transfection and infection efficiency was observed under an inverted fluorescent microscope.
Confocal laser scanning fluorescence microscopy
FLSs were cultured on coverslips under identical conditions to those described above. The cells were fixed with acetone at −20°C and permeabilized with 0.1% Triton X-100 in PBS for 5 min at room temperature. The cells were incubated with primary antibodies (diluted 1:100 for anti-p65 antibody, diluted 1:200 for Brd2, Brd3, Brd4 and Brdt antibodies) for 1 h at room temperature, and then incubated with fluorescein isothiocyanate-conjugated secondary antibody (Santa Cruz Biotechnology). After being washed in PBS, the cells were incubated for 3 min with 0.25 mg/ml of 4′, 6-diamidino-2-phenylindole. The coverslips were mounted on glass slides with anti-fade mounting media and examined using a confocal fluorescence microscope (LSM510; Zeiss, Wetzlar, Germany).

Western blot analysis
Analyses of the cells by western blotting were performed as described previously [20]. The protein concentrations were measured using a BCA protein assay (Pierce, Rockford, IL, USA). The primary antibodies were diluted 1:1000 for IkBa, p65, Brd2, Brd3, Brd4 and Brdt antibodies) for 1 h at room temperature, and then incubated with fluorescein isothiocyanate-conjugated secondary antibody (Santa Cruz Biotechnology). After being washed in PBS, the cells were incubated for 3 min with 0.25 mg/ml of 4′, 6-diamidino-2-phenylindole. The coverslips were mounted on glass slides with anti-fade mounting media and examined using a confocal fluorescence microscope (LSM510; Zeiss, Wetzlar, Germany).

NF-κB reporter assay
FLSs were transiently transfected with 1 μg of pNF-κB-Luc plasmid, using Lipofectamine reagent according to the instructions of the manufacturer (Invitrogen). Twenty-four hours later, the transfected cells were starved overnight in a serum-free medium and then seeded in 96-well plates. The cells were then treated with various agents and harvested in reporter lysis buffer (Promega, Madison, WI, USA). After normalization of transfection efficiency by the β-galactosidase expression, the luciferase enzyme activity was quantified using a reporter assay kit (Clontech, Palo Alto, CA, USA).

Measurement of pro-inflammatory cytokine and MMPs production
Cell culture supernatants were collected for the measurement of TNFα, IL-1β, IL-6, IL-8, MMP-1, MMP-3 and MMP-13 by ELISA using a commercial kit, according to the instructions of the manufacturer (R&D Systems, USA).

Quantitative real-time PCR analysis
The total RNA from RA FLSs was extracted using an RNeasy Mini kit (Qiagen). Quantitative real-time PCR was performed using a Qiagen SYBR Green PCR kit with a 7300 real-time PCR system (ABI). The relative mRNA expression levels were determined using the difference in threshold cycles for target and reference values and normalized using housekeeping gene glyceraldehyde-3-phosphate dehydrogenase. The RT-PCR primers used were listed in Supplementary Table S2, available at Rheumatology online.

Measurement of RA FLSs proliferation
RA FLSs were cultured for 24 h at a density of 1 × 10⁴/well in 96-well plates in serum-free medium. After starving, the cells were incubated with TNFα (10 ng/ml) for 72 h and then incubated with 10 mM BrdU for 1 h. BrdU incorporation was assessed in triplicate, using a cell proliferation ELISA kit (Roche, Mannheim, Germany) according to the manufacturer’s instructions.

Determination of JQ1 on CIA
CIA was induced in DBA1 mice by intradermal injection of 200 μg of bovine type II collagen emulsified at a 1:1 ratio

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P: positive; N: negative.
(vol/vol) in Freund’s complete adjuvant, and then boosted 3 weeks later using bovine type II collagen emulsified at a 1:1 ratio (vol/vol) in incomplete Freund’s adjuvant. Disease onset characterized by erythema and/or paw swelling was seen from day 32 to 41 after primary immunization. Animals were treated with intraperitoneal injection of dimethyl sulfoxide (DMSO) (vehicle, twice daily) or JQ1 (30 mg/kg, twice daily) for a total of 14 days, initiated on the day of arthritis onset (day 0). After 14 days treatment with JQ1, the animals continued to be observed for 7 days (without treatment with JQ1 or DMSO). The mice were monitored daily for signs of arthritis, and arthritis severity was scored on a scale of 0–3, as described previously [22]. The total score was recorded as the sum of the scores in the four limbs. The hind limbs were removed and fixed in 10% neutral buffered formalin. The tissue sections (3 µm) were stained with haematoxylin and eosin. An inflammation score was obtained, using the scoring system described previously [22].

For determining the toxic effect of JQ1 on mice with CIA, biochemical analyses of serum samples were determined by the colorimetric enzymatic method using a spectrophotometric technique. The enzymatic activities of aspartate aminotransferase and alanine aminotransferase were detected to investigate liver function alterations. Serum creatinine was detected as a renal parameter. These parameters were analysed using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions. Furthermore, we also observed histopathology of liver and kidney by staining with haematoxylin and eosin.

Measurement of serum cytokines production and IgG antibody to type II collagen

Serum levels of IgG antibody to type II collagen (anti-CII antibody) and pro-inflammatory cytokines (IL-6 and TNF-α) 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.

Statistical analysis

The data are expressed as the mean (S.E.M.). Analysis of variance with the Student–Newman–Keuls test was used to evaluate the differences between the experimental groups. The histological results were analysed by the Kruskal–Wallis non-parametric test. P < 0.05 was considered significant.

Results

Expression patterns of Brd proteins in FLSs from patients with RA

We first evaluated the expression patterns of BETs in FLSs. Western blot analysis revealed that all of the four BET proteins, including Brd2, Brd3, Brd4 and Brdt, were expressed in FLSs from patients with RA and OA. We found that the expression of Brd2 and Brd4 was increased in RA compared with that in OA; however, the expression of Brd3 and Brdt were equivalent in the samples (Fig. 1A).

We further evaluated the difference in Brd proteins expression in FLSs from RA patients between untreated patients and patients treated with prednisone and/or DMARDs, and found that the expression of all of the BET proteins in RA FLSs did not differ between them (Fig. 1B). We also found that Brd2, Brd3 and Brdt proteins localized in the nucleus as well as in the cytoplasm, and that Brd3 was prominent in the cytoplasm, whereas Brdt was predominantly in the nucleus. However, Brd4 was located completely in the nucleus (Fig. 1C).

BET inhibition suppresses production of pro-inflammatory cytokines in RA FLSs

JQ1 is a broad-spectrum BET protein inhibitor that suppresses interaction between multiple BET proteins (Brd2/3/4) and acetylated histones [23]. Therefore, we used JQ1 as a specific BET inhibitor. As shown in Fig. 2, LPS-stimulated TNF-α secretion, as well as TNF-α-induced IL-6, IL-1β and IL-8 secretions, were reduced by treatment with JQ1 in concentrations ranging from 0.1 to 0.5 µM. The viability of RA FLSs was measured by the MTT test to evaluate the toxic effect of JQ1 on RA FLSs. Up to 1 µM, this compound did not reduce cell viability (data not shown), which indicates that the observed inhibitory effects were not caused by cytotoxic effects.

To further rule out a non-specific effect of the small molecule inhibitor, we utilized the RNA interference technique to selectively reduce Brd expression. FLSs were transfected with Brd2 shRNA, Brd4 shRNA or the control shRNA. Compared with cells transfected with the control shRNA, the Brd2 shRNA or Brd4 shRNA markedly suppressed the endogenous protein expression of Brd2 or Brd4 (data not shown). After 72 h of transfection, the cells were treated with LPS or TNF-α for 24 h. Transfection with shRNA directed against Brd2 or Brd4 decreased the LPS- or TNF-α-induced cytokine production compared with the control shRNA treatment (Fig. 2) and confirmed our results obtained with JQ1.

BET inhibition suppresses MMPs production and proliferation in RA FLSs

MMPs play important roles in synovial inflammation and joint destruction. We found that TNF-α-induced mRNA expression and secretion of MMP-1, MMP-3 and MMP-13 was decreased by JQ1 in concentrations ranging from 0.1 to 0.5 µM. Transfection with Brd2 shRNA or Brd4 shRNA also decreased TNF-α-induced mRNA expression and secretion of MMP-1, MMP-3 and MMP-13 (Fig. 3A).

Previous studies indicate that BET inhibition suppresses tumor cell proliferation. To address whether BET Brd regulates proliferation of RA FLSs, the cells were treated with TNF-α (10 ng/ml) and BrdU (10 mM) in the presence or absence of JQ1. We showed that JQ1 treatment, as well as transfection with Brd2 shRNA or Brd4 shRNA, reduced TNF-α-induced proliferation of FLSs (Fig. 3B).
BET inhibition prevents NF-κB activation through IκB kinase in RA FLSs

To determine whether BET inhibition affects NF-κB activation, we first evaluated the effect of JQ1 on NF-κB gene transcription by using NF-κB/C150-dependent luciferase gene reporter plasmid. As shown in Fig. 4A, JQ1 treatment significantly decreased the NF-κB/C150-dependent transcription of a luciferase reporter gene induced by TNFα. To confirm the inhibitory effect of JQ1 on NF-κB gene transcription, we determined the expression of the TNFα-induced NF-κB target gene of RA FLSs in the presence of JQ1. When RA FLSs were treated with JQ1 and stimulated with TNFα for 8h, we found that TNFα induced the expression of a subset of NF-κB target genes, including IL-6 and IL-8, that were downregulated by JQ1 (Fig. 4B). Additionally, we demonstrated that transfection with Brd2 shRNA or Brd4 shRNA reduced the NF-κB-dependent transcription of a luciferase reporter gene and expression of target genes (Fig. 4A and B). These data indicate that BET inhibition prevents TNFα-induced NF-κB activation.

In our effort to explore the mechanisms of BET inhibition on NF-κB activation, we found a significant decrease in phosphorylated IκBα following treatment with JQ1 in TNFα-stimulated RA FLSs (Fig. 3C). Consistent with the decreased IKK activity, JQ1 treatment inhibited TNFα-induced phosphorylation and degradation of IκBα.
We also observed a significant reduction in p65 nuclear accumulation in the RA FLSs treated with JQ1 compared with the cells treated only with TNFα (Fig. 4D). Furthermore, we observed that transfection with Brd2 shRNA or Brd4 shRNA suppressed the TNFα-induced IKK activity (Fig. 4E), excluding the off-target inhibition of BET proteins function. These data suggest that BET inhibition suppresses NF-κB activation through IKK signalling.

BET inhibition with JQ1 attenuates synovial inflammation and suppresses serum anti-CII antibodies and cytokines in mice with CIA

Given these observations in cultured RA FLSs, the in vivo effect of BET inhibition in synovial inflammation of RA was assessed in mice with CIA. Previous studies have shown that JQ1 potently suppress BET function in adult mice without significant toxicity when administered chronically at 50–100 mg/kg/day [23, 24], and exhibits even more excellent effects with twice-daily administration [23]; therefore, the animals were treated with a twice-per-day dose of 30 mg/kg of JQ1 in our experiments. Intraperitoneal injection of JQ1 suppressed the increase in the clinical score compared with that of animals treated with DMSO (Fig. 5A–C). The DMSO–treated mice demonstrated that inflammation occurred in the synovial, subsynovial and periosteal tissue and was characterized by oedema and inflammatory cells infiltrate; the synovial membranes were generally degenerate and necrotic, with areas of hyperplasia, and profound cartilage surface erosion.
RA FLSs were pretreated with DMSO, as the control, or various concentrations of a specific BET inhibitor, JQ1, for 3 h or transfected with specific shBrd2 or shBrd4 or shC and then stimulated with or without TNFα (10 ng/ml) for 12 (for MMPs mRNA) or 24 (for MMPs secretion) or 48 (for proliferation) h. (A) Effect of BET inhibition on MMPs expression. mRNA expression of MMP-1, MMP-3 and MMP-13 (upper panel) was determined by quantitative real-time PCR. Data were normalized to glyceraldehyde-3-phosphate dehydrogenase. The levels of the MMP-1, MMP-3 and MMP-13 (lower panel) in cultured cell supernatants were measured by ELISA. (B) Effect of BET inhibition on RA FLSs proliferation, measured by BrdU incorporation into cell DNA. Data represent the mean ± S.E.M. of five independent experiments from five different RA patients *P < 0.05 vs DMSO or shC #P < 0.05 vs TNFα or shC+ TNFα. FLSs: fibroblast-like synoviocytes; BET: bromodomain and extra-terminal domain; DMSO: dimethyl sulfoxide; shBrd2: Brd2 shRNA; shBrd4: Brd4 shRNA; shC: control shRNA; Brd: bromodomain; RLU: relative light unit.
**Fig. 4** Effects of BET inhibition on TNF-α-induced NF-κB activation in RA FLSs

(A) The cells were transfected with NF-κB luciferase gene reporter plasmids or co-transfected with shBrd or shC or pretreated with JQ1, and then were stimulated with TNF-α (10 ng/ml) for 6 h. The data shown represent NF-κB luciferase activity (relative light unit, mean ± S.E.M.) from five RA patients. (B) RA FLSs, pretreated with JQ1 for 3 h or transfected with shBrd or shC, were stimulated with TNF-α (10 ng/ml) for 24 h. Quantitative real-time PCR was performed to analyse the expression of NF-κB target genes (IL-6 and IL-8). (C) Effect of JQ1 on IKK and IκB phosphorylation. The lower panel shows a densitometric analysis of western blots. (D) Representative laser confocal microscopy images show effect of JQ1 on TNF-α-induced translocalization of NF-κB p65 (red stain) with nuclei stained with DAPI (blue stain). (E) Effects of shBrd transfection on the IKK phosphorylation. *P < 0.05 vs DMSO or shC, †P < 0.05 vs TNF-α or shC + TNF-α. Fibroblast-like synoviocytes; BET: bromodomain and extra-terminal domain; DMSO: dimethyl sulfoxide; shBrd: Brd shRNA; shBrd4: Brd4 shRNA; shC: control shRNA; shBrd2: Brd2 shRNA; shBrd4: Brd4 shRNA; IKK: IκB kinase β; pIKK: phosphorylated IκB kinase β; DAPI: 4′, 6-diamidino-2-phenylindole; Brd: bromodomain.
was observed. These signs of inflammation and cartilage erosion were decreased significantly in the JQ1-treated animals (Fig. 5D, upper panel), and the macroscopic inflammation score was reduced accordingly (Fig. 5D, lower panel).

Moreover, the levels of anti-CII antibodies in the sera of mice with CIA were higher than the levels of normal healthy mice. JQ1 treatment resulted in a significant reduction in the level of total anti-CII IgG in mice with CIA. The levels of anti-CII IgG2a and IgG1 were also markedly reduced.
decreased by JQ1 (Fig. 5E). We also observed a significant reduction in the levels of serum IL-6 and TNF-α in the JQ1-treated mice with CIA (Fig. 5F).

No significant gain in body weight was seen between the JQ1 and DMSO groups over the course of the experiment. We found no significant changes in the renal (serum creatinine level) or liver (alanine aminotransferase and aspartate aminotransferase levels) parameters in mice treated with JQ1 (supplementary Table S3, available at Rheumatology online). Furthermore, we also found no significant histopathological alterations of livers and kidneys removed from JQ1-treated mice as compared with DMSO-treated mice (Fig. 5G). These data suggest the safety of JQ1 treatment in CIA mice.

Discussion

This work shows that BET Brd inhibition downregulates cytokines production, MMPs expression and proliferation of RA FLSs. More importantly, we demonstrated the inhibitory effect of BET inhibition on activation of NF-κB through blocking the IKK signal. BET inhibitor JQ1 markedly reduces the severity of synovial hyperplasia, inflammatory cell infiltration and joint destruction in mice with CIA. Our findings strongly suggest that BET inhibition, targeting NF-κB-mediated synovial inflammation, represents a new therapeutic strategy for RA.

Pro-inflammatory cytokines, particularly TNF-α, IL-1β and IL-6, play a critical role in synovial inflammation in RA. How to regulate the abnormal production of a series of pro-inflammatory cytokines in RA remains unclear. Increasing evidence shows that chromatin readers, which specifically bind histone post-translational modifications and provide a framework of an integral element of the transcriptional activation complex, play important roles in modulating inflammatory responses to a variety of immune system stimuli [25–27]. As Brd-containing reader proteins, Brd2 and Brd4 are involved in chromatin acetylation and promote inflammatory transcriptional activation [14, 28–30]. Recent studies indicate that BET proteins might be associated with immune and inflammatory responses. For instance, BET inhibition results in a variety of alterations in T cell cytokine production [31], and suppresses differentiation and activation of Th17 cells [32]. BET Brd also regulates the LPS-induced expression of pro-inflammatory cytokines and chemokines in murine macrophages [15].

In this work, we attempted to elucidate the role of BET Brd proteins in FLSs-mediated synovial inflammation in RA. Our results showed that the expression of Brd2 and Brd4 was increased in RA FLSs compared with in OA FLSs. BET inhibition could suppress not only the production of TNF-α, IL-1β, IL-6 and IL-8, but also the expression of MMP-1, MMP-3 and MMP-13 and proliferation in RA FLSs, suggesting the role of BET in regulating FLSs-mediated inflammation. The results from in vitro experiments that JQ1 attenuates synovial inflammation and joint destruction in mice with CIA further support the notion that BET proteins are involved in the synovial inflammation in RA, indicating that BET Brd might be a new target for prevention of the synovial inflammatory process of RA. Consistent with our findings, previous work has identified the inhibitory effect of JQ1 in IL-6 expression in neonatal rat ventricular cardiomyocytes [33].

As a critical transcriptional factor in regulating the expression of a great number of pro-inflammatory genes, NF-κB is considered as a key signalling factor in controlling synovial hyperplasia, inflammation and matrix degeneration [7, 8]. To investigate the mechanisms for BET Brd in regulating synovial inflammatory responses, we observed the effect of BET inhibition on NF-κB activation. We demonstrated that BET inhibition with JQ1 or Brd shRNA suppressed TNF-α-induced NF-κB-dependent transcription of a luciferase reporter gene and NF-κB target genes, indicating the involvement of the BET Brd in NF-κB activation by RA FLSs. Consistent with our data, recent studies have shown that BET Brd inhibition can regulate transcription activation of NF-κB target genes in macrophages [15], renal tubular epithelial cells [34] and tumor cells [35, 36].

NF-κB activation could be modulated at multiple steps. Based on the previous studies in macrophages [15] and HIV-associated kidney disease [34], we hypothesize initially that BET proteins contribute to synovial inflammation due to a direct effect in NF-κB transcriptional activity. However, we observed unexpectedly that BET inhibition suppresses TNF-α-induced phosphorylation of IKKβ and IκBα, and translocation of nuclear NF-κB, indicating that the nuclear BET proteins regulate the NF-κB pathway through interfering in early cytoplasmic IKK signalling. A recent work also shows that BET inhibitors block oncogenic IKK activity in diffuse large B-cell lymphoma [37]. In addition, we also found that BET inhibition reduced the cytoplasmic phosphorylation of the p38 MAPK pathway in TNF-α-stimulated RA FLSs (Xiao et al., unpublished data). These findings indicate that BET proteins can affect cytoplasmic signalling through some kind of unknown mechanisms. It will be important to explore the detailed mechanisms with which BET proteins modulate cytoplasmic IKK activity in future study.

In summary, we demonstrate that BET proteins play an important role in modulating the arthritic inflammatory response by inhibiting NF-κB activation via blocking IKK signalling, and suggest that targeting epigenetic regulators such as BET proteins might be a potential therapeutic strategy for inflammatory arthritis.

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Supplementary data

Supplementary data are available at Rheumatology online.

References


