JQ1, a BET inhibitor, controls TLR4-induced IL-10 production in regulatory B cells by BRD4-NF-κB axis

Min Bum Lee1,#, Jun-Ho Lee2,#, Seong Hwi Hong1, Jueng Soo You1, Seung Taek Nam1, Hyun Woo Kim1, Young Hwan Park1, Dajeong Lee1, Keun Young Min1, Yeong-Min Park1, Young Mi Kim3, Hyuk Soon Kim1,* & Wahn Soo Choi1,*

1School of Medicine, Konkuk University, 2Department of Biomedical Chemistry, College of Biomedical & Health Science, Konkuk University, Chungju 27478, 3College of Pharmacy, Duksung Women’s University, Seoul 01369, Korea

INTRODUCTION

Regulatory B cells, also well-known as IL-10-producing B cells, play a role in the suppression of inflammatory responses. However, the epigenetic modulation of regulatory B cells is largely unknown. Recent studies showed that the bromodomain and extra-terminal domain (BET) protein inhibitor JQ1 controls the expression of various genes involving cell proliferation and cell cycle. However, the role of BET proteins on development of regulatory B cells is not reported. In this study, JQ1 potently suppressed IL-10 expression and secretion in murine splenic and peritoneal B cells. While bromodomain-containing protein 4 (BRD4) was associated with NF-κB on IL-10 promoter region by LPS stimulation, JQ1 interfered the interaction of BRD4 with NF-κB on IL-10 promoter. In summary, BRD4 is essential for toll like receptor 4 (TLR4)-mediated IL-10 expression, suggesting JQ1 could be a potential candidate in regulating IL-10-producing regulatory B cells in cancer. [BMB Reports 2017; 50(12): 640-646]

Keywords: BRD4, IL-10, JQ1, NF-κB, Regulatory B cells

*These authors contributed equally to this work.

https://doi.org/10.5483/BMBRep.2017.50.12.194

Received 13 October 2017, Revised 30 October 2017, Accepted 28 November 2017

Copyright © 2017 by the The Korean Society for Biochemistry and Molecular Biology

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
results suggest that JQ1 can be used as a novel therapeutic molecule for anti-cancer immunity targeting regulatory B cells.

RESULTS

LPS-stimulated IL-10 production is controlled by JQ1 in B cells

First, the expression of IL-10 gene in splenic B cells was confirmed by lipopolysaccharide (LPS) stimulation. IL-10 gene expression in splenic B cells was increased in response to LPS stimulation in a time- and dose-dependent manner (Fig. 1A and B). It was also found that IL-10 secretion by splenic B cells was also increased by LPS stimulation (Fig. 1C). Next, the expression of IL-10 in B cells by JQ1, a specific inhibitor of BRD4, was examined. IL-10 gene expression decreased in a dose-dependent manner when splenic B cells stimulated with LPS were treated with JQ1 (Fig. 1D and E). The concentration of JQ1 treatment in vitro were determined at the preliminary experiment (Supplementary Fig. 1), and the JQ1-mediated regulation of IL-10 gene expression and secretion by LPS was not due to cytotoxicity (Fig. 1F).

Fig. 1. LPS-induced IL-10 expression is regulated by JQ1 in B cells. (A) Representative images and relative gene expression of IL-10. Splenic B cells were stimulated with LPS (10 μg/ml) for indicated times or (B) indicated LPS concentration for 4 h. Representative band images and data are the mean ± SEM from three independent experiments. **P < 0.01. (C) Quantitative analysis of secreted IL-10 from LPS-stimulated splenic B cells. Cells were stimulated with LPS for the indicated times and supernatant were harvested. IL-10 released into the medium were analyzed by enzyme-linked immunosorbent assay (ELISA). **P < 0.01 (24 h) or **P < 0.01 (48 h) when compared with no LPS stimulation group. (D) Representative images and (E) relative gene expression of IL-10 gene expression in LPS stimulated splenic B cells with or without JQ1 (0-100 nM) for 4 h. (F) Splenic B cells were incubated in triplicate LPS with or without JQ1 (0-100 nM) for 24 h. Cell viability was determined using a cell counting kit-8. Representative band images (D) and data (E, F) are the mean ± SEM from three independent experiments. **P < 0.01. n.s., not significant.

Fig. 2. Effect of JQ1 on regulatory B subsets and IL-10 producing ability in splenic and peritoneal cavity B cells. (A) Expressions of the B cell surface markers from DMSO or JQ1 (100 nM) treated splenic B cells that were incubated with LPS for 4 h. Black histograms represent B cells treated with DMSO (control), red histograms represent JQ1-treated B cells, and gray filled histograms show cells with isotype control. Plots are representative images of at least three independent experiments. (B and C) Splenic B cells were incubated with 10 μg/ml of LPS + PIM for 5 h in presence or absence of the indicated dose of JQ1. (B) Representative plots and frequencies of CD1dhiCD5+ subset and (C) IL-10+ in splenic B cells. **P < 0.01. n.s., not significant. (D) Frequencies of IL-10+ splenic B cells that contained LPS for 43 h + last PIM 5 h in the presence of the indicated dose of JQ1. *P < 0.05, **P < 0.01. (E) Secretion of IL-10 in LPS stimulated splenic B cells with or without JQ1 (0-100 nM) for 24 and 48h. *P < 0.05, **P < 0.01 (24 h) or **P < 0.01 (48h) when compared with JQ1 untreated B cells under LPS stimulation group. (F and G) Peritoneal B cells were incubated with 10 μg/ml of LPS + PIM for 5 h in presence or absence of the indicated dose of JQ1. (F) Representative plots and frequencies of CD5+CD11b- B-1a cells and (G) IL-10+ in peritoneal cavity B cells. *P < 0.05, **P < 0.01, n.s., not significant. (H) Frequencies of IL-10+ peritoneal cavity B cells that contained LPS for 43 h + last PIM 5 h in the presence of the indicated dose of JQ1. *P < 0.05, **P < 0.01, n.s., not significant. (I) Secretion of IL-10 in LPS stimulated peritoneal cavity B cells with or without JQ1 (0-100 nM) for 24 and 48 h. *P < 0.05, **P < 0.01 (24 h) or **P < 0.01 (48 h) when compared with JQ1 untreated B cells under LPS stimulation group. All plots are representative images and data are the mean ± SEM from three independent experiments.
BRD4 pathway is associated with the development of IL-10-producing B cells

We observed that the treatment of JQ1 did not change the phenotype of B cells (Fig. 2A). It is well known that the frequency of IL-10-producing B cells is closely related to the splenic CD1d<sup>+</sup>CD5<sup>+</sup> phenotype (12). We next checked whether the decrease in IL-10 production function of B cells by JQ1 correlates with the decrease of CD1d<sup>+</sup>CD5<sup>+</sup> B cells. Unexpectedly, there was no frequency change of CD1d<sup>+</sup>CD5<sup>+</sup> B cells by a JQ1 treatment (Fig. 2B). Flow cytometry analysis showed that IL-10 production in LPS-induced B cells decreased in a dose-dependent manner (Fig. 2C). In previous study, Tedder and coworkers reported that precursor cells of regulatory B cells can differentiate into mature regulatory B cells by long-term stimulation of LPS (26). The effect of JQ1 on the differentiation of precursor cells by long-term LPS stimulation was also investigated. JQ1 reduced the differentiation of precursor cells to mature IL-10-producing regulatory B cells (Fig. 2D and E).

The major population of regulatory B cells in the peritoneal cavity (PeC) has the phenotype of CD5<sup>+</sup>CD11b<sup>+</sup> (B-1a). The changes of CD5<sup>+</sup>CD11b<sup>+</sup> regulatory B cells by JQ1 were examined and it was found not to induce any phenotypic change as in splenic regulatory B cells (Fig. 2F). Consistently, the differentiation of precursor cells of PeC regulatory B cells by the long-time treatment of LPS and the IL-10 secretion of PeC B cells were also suppressed by JQ1 (Fig. 2H and I). Of note, the production of IL-10 in splenic
CD1dCD5^- and PeC CD5^-CD11b^- B cell subsets, but not the other subsets, were suppressed by JQ1 (Supplementary Fig. 2). These results suggest that the BRD4 pathway affects LPS-induced IL-10 production in spleen and peritoneal cavity but not regulatory B subset frequency. Therefore, we hypothesized that the BRD4 pathway is associated with the signal transduction pathway for the production of IL-10 in regulatory B cells.

**LPS treatment does not affect expression of BET proteins and NF-kB in B cells**

Next, we investigated whether LPS stimulation can change the expression of BET proteins themselves. As a result, any change of BET proteins was not observed in splenic B cells by stimulation of LPS (Fig. 3A to D). The expression of NF-kB p65 was also not changed by the treatment of LPS (Fig. 3E and F). Additionally, we hypothesized that the suppression of IL-10 production from B cells by JQ1 is associated with the expression of NF-kB p65. We then found that JQ1 did not alter the expression level of NF-kB p65 (Fig. 3G), suggesting that the negative mechanism of JQ1 is not directly associated with the levels of NF-kB p65 in the process of IL-10 production in regulatory B cells.

**JQ1 inhibits the recruitment of BRD4 and NF-kB p65 at IL-10 promoter region upon LPS stimulation**

In the TLR signal transduction pathway by LPS stimulation, several reports demonstrated that the multiple chromatin complex with NF-kB p65 are formed at IL-10 DNA regulatory regions (27, 28). Besides, NF-kB was also known to interact with BRD4 in other cells (29). Based to these, we designed three sites for IL-10 DNA regulatory regions covering the hyper sensitive site (HSS, upstream 4.5 kb) (30), distal promoter (DP, upstream 1.2 kb), and proximal promoter (PP, upstream 0.2 kb) (31, 32) which are known as functional DNA elements for IL-10 transcription (Fig. 4A). We then performed chromatin immunoprecipitation (ChIP) assay with antibody against BRD4 and NF-kB p65 in splenic B cells. We found that NF-kB p65 binding, but not BRD4, was increased at two promoter sites by LPS (Fig. 4C and D). Interestingly, the ChIP analysis revealed significant increase in binding of BRD4 on IL-10 proximal promoter among three sites where the greater NF-kB p65 binding was observed concurrently and these recruitments suppressed by JQ1 (Fig. 4C). Therefore, we argue that BRD4 may be critical for NF-kB p65 binding on IL-10 proximal promoter for the production of IL-10 in regulatory B cells.

**DISCUSSION**

In the past decade, the suppressive effects, mainly through the secretion of IL-10, of regulatory B cells on inflammatory responses have been reported in a variety of immune disorders (33-36). Additionally, immune regulation through the interaction of immune cells with the intrinsic phenotype of regulatory B cells (e.g., CD1dCD5^-, T2-MZP, Tim-1^-, and CD9^+) were demonstrated in various diseases, and it plays a critical role in autoimmune diseases (37). In recent studies, functional studies in cancer diseases are emerging (38-40). In particular, the change of the distribution of regulatory B cells in cancer tissue is considered to one of important indicators (8-10). Emerging evidence suggests that regulatory B cells suppress effector immune cells including IFN-γ-producing cytotoxicity cells in various cancer diseases through the secretion of IL-10 (11). Although regulatory B cells have to play the suppressive role on the effector function of T cells in autoimmune diseases to cure diseases (41), regulatory B cells need to be suppressed to induce anti-cancer immunity to cure cancer.

As development of BET protein inhibitors, there are tremendous effort to apply these drug to various fields such as cancer and immune disorders. In cancer, they are well known as pivotal regulators for the expression of several oncogenes, such as c-Myc and Bcl-2 (42, 43). Moreover, several BET protein inhibitors have been under clinical research (44). However, there is no study on the relationship between BET proteins and regulatory B cells, especially for IL-10 production. Furthermore, the epigenetic mechanism the production of IL-10 in the nucleus of regulatory B cells is largely unknown. This study proposes BRD4, a chromatin reader is a critical modulator of regulatory B cells. We found that the gene expression and secretion of IL-10 by LPS stimulation were reduced in regulatory B cells by the treatment of JQ1 in a dose-dependent manner (Fig. 1). The effect of JQ1 is not due to the induction of changes of regulatory B cell phenotype but modulation of the IL-10 production in regulatory B cells (Fig. 1G and Fig. 2).

It is generally accepted that LPS-mediated TLR4 signal pathway is critical to increase the frequency of IL-10 producing regulatory B cells. However, the epigenetic regulation on the production of IL-10 in regulatory B cells by LPS is not well known. It has reported that BRD4 is interacted with various proteins including several transcription factors. Among them, NF-kB is critical for the production of IL-10 (27-29). In this study, we examined whether the relationship between BET proteins and NF-kB (a major transcription factor for IL-10 production by LPS) were altered by LPS stimulation. The expression of total BRD4 (also BRD2 and BRD3) and NF-kB p65 proteins was not affected by LPS stimulation or JQ1 treatment in B cells (Fig. 3A to G). These results suggest that BRD4 may be directly involved in transcriptional activation IL-10 via the TLR4 signal by LPS. Therefore, we further assessed the role of BRD4 in LPS-mediated IL-10-producing B cells by using ChIP assay. Three major sites such as hyper sensitive site (HSS), distal promoter (DP), and proximal promoter (PP) were investigated by ChIP assay. LPS stimulation caused recruitment of BRD4 and NF-kB to the IL-10 proximal promoter region of B cells, and this process was inhibited by JQ1 (Fig. 4D), suggesting

http://bmbreports.org

JQ1 suppresses regulatory B cells activation

Min Bum Lee, et al.

BMB Reports 643
that BRD4 play critical role for production of IL-10 of regulatory B cells. Although it was reported that BRD4 directly binds to acetylated NF-kB p65 in LPS stimulation (45), whether both proteins are directly interacted in regulatory B cells remained to be determined.

In summary, this study demonstrates that BRD4 as a novel epigenetic regulator directly participates in the transcriptional process for IL-10 production via altering chromatin structure in regulatory B cells upon LPS simulation and presumably this mechanism could contribute anti-cancer effects of JQ1 in various cancer diseases.

MATERIALS AND METHODS

Mice

C57BL/6 (5-6-week-old male) mice were purchased from Orient Bio Inc. (Gyeonggi, Korea). Mice were housed under specific pathogen free facility at Konkuk University (Seoul, Korea). The animal study was done in accordance with the institutional guidelines. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Konkuk University.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Splenic B cells were purified with CD19 mAb-conjugated microbeads (Miltenyi Biotech, Auburn, CA) according to the manufacturer’s instructions, to >95% purity. Total RNA was isolated from sorted Splenic CD19+ B cells (1 × 10⁶ cells) by using easy-BLUE (iNtRON Biotechnology, Gyeonggi, Korea) and reverse-transcribed with the ImProm-II reverse transcription first-strand synthesis system (Promega, Madison, WI) according to the manufacturer’s protocol. PCR was performed using easy-BLUE (iNtRON Biotechnology, Gyeonggi, Korea) and reverse-transcribed with the ImProm-II reverse transcription first-strand synthesis system (Promega, Madison, WI) according to the manufacturer’s protocol. PCR was performed at 95°C for 2 min, 30 cycles of 95°C for 20 sec, 58°C for 40 sec, 72°C for 30 sec, and 72°C for 5 min. Primers were used as follows: mouse BRD2 (forward 5'-CCACGAAAAAGACTTGGCC TGA-3', reverse 5'-CCAGCTGTCTTTGGAGACG-3'); mouse BRD3 (forward 5'-CTATGGTCGGGCCCTTTGTA-3', reverse 5'-CTGCCCTGATTCTGCTGA-3'); mouse BRD4 (forward 5'-CAAAAAGAAAGAGGACGAGG-3', reverse 5'-ACAGGTG GAGGGGTTCTGTG-3'); mouse IL-10 (forward 5'-GGCCTCAG AAATCGAAGCAGGA-3', reverse 5'-GGGGGATGACAGTAGGG GAA-3'); mouse GAPDH (forward 5'-GGCGGCGGCCCTTGA GAAA-3', reverse 5'-AGTGTAGCCAAAAATCATTG-3').

Measurement of Interleukin-10 release by ELISA

Isolated splenic CD19+ B cells (3 × 10⁶ cells/well) and Peritoneal cavity fluid (PeC)-derived CD19+ B cells (1 × 10⁶ cells/well) were stimulated with LPS (0, 0.1, 1, and 10 μg/ml) for 24 or 48 h, the level of IL-10 by using a mouse BD OptEIA IL-10 ELISA kit according to the manufacturer’s instructions (BD Biosciences, San Jose, CA).

Flow cytometry analysis

Splenic and PeC CD19+ B cells (3 × 10⁶ cells/well) stimulated for 5 or 48 h with LPS (10 μg/ml, Sigma-Aldrich, St. Louis, MO). The B cells were incubated with LPS alone or with JQ1 (0, 20, 50, and 100 nM) for 5 or indicated times in figure legends, and phosphor 12-myristate 13-acetate (PMA, 50 ng/ml, Sigma-Aldrich), ionomycin (500 ng/ml, Sigma-Aldrich), and Monensin (2 μM, eBioscience, San Diego, CA) were added during last 5 h. Prior to surface staining, Fcγ receptors were blocked with anti-CD16/CD32 monoclonal antibodies (2.4G2, BD Biosciences). Cells were fixed and permeabilized with a Cytofix/Cytoperm kit (eBioscience) and then were stained with anti-IL-10 (JES5-16E3, eBioscience) (46). The antibodies against surface proteins were as follows: CD1d (1B1), CD5 (53-7.3), CD11b (M1/70), CD19 (eBio1D3), CD21/CD33 (eBioBD9), CD23 (B3B4), CD40 (HM40-3), CD86 (GL1), B220 (RA3-6B2), IgM (eB121-15F9), IgD (11-26), MHCI (M5/114.15.2), all purchased from eBioscience. Cells were analyzed with FACS Canto II (BD Biosciences, San Jose, CA) and FlowJo V10 software (TreeStar, Ashland, OR). The gate strategy for IL-10-producing B cells was illustrated in Supplementary Fig. 3.

Measurement of cell viability

Mouse splenic CD19+ B cells (3 × 10⁶ cells/well) were plated on 24-well plates in LPS contained media with or without JQ1 for 24 h. Then the cell viability was determined by using a cell counting kit-8 (CCK-8, Dojindo Lab, Kumamoto, Japan), according to the manufacturer’s protocol.

Immunoblotting

Splenic CD19+ B cells (1 × 10⁷ cells/well) were lysed in RIPA buffer containing protease inhibitor on ice for 10 min. Lysate centrifuged at 13,000 × g for 10 min at 4°C. Protein lysates of each sample were analyzed by western blotting using specific antibodies (47). Antibodies against NF-κB p65 and actin were purchased from Cell Signaling Technology (Danvers, MA, USA). Immunoreactive proteins were detected with HRP-coupled secondary antibodies and enhanced chemiluminescence according to the manufacturer’s protocol (Amersham Biosciences, Piscataway, NJ).

Chromatin immunoprecipitation (ChIP)

ChIP was performed with the splenic B cells following to instructions from Upstate Biotechnology (Lake Placid, NY, USA). Cells (2 × 10⁶ cells/well) were harvested from the culture and fixed with 37% formaldehyde for 10 min. For each assay, sheared by a sonication (the DNA fragment size was 200 to 400 bp), was precleared with protein A magnetic beads and then 50 μg DNA was precipitated by BRD4 (Bethyl Laboratories, Montgomery, TX) or NF-kB p65 (Cell Signaling Technology). After immunoprecipitation, recovered chromatin fragments were subjected to real-time PCR. IgG control experiments were performed for all ChIPs and incorporated into the IP/Input (1%) by presenting the results as (IP-IgG/Input-IgG). The primers used as follow: ChIP IL-10 hyper sensitive site (forward 5'-GGCGGAATATACACCTATGTC-3',
reverse 5'-CCGGATTGAGTCTCCTGAG-3'); ChIP IL-10 Distal Promoter (forward 5'-CCCTGCTGTGGTGAACCTCT-3', reverse 5'-ACCTGGCAGCAGCACTACT-3'); ChIP IL-10 Proximal Promoter (forward 5'-GCAGAAGTTCATTCCGACCA-3', reverse 5'-GCCTTTGGCCTTTGGTAGTG-3').

Statistical analysis
The data are expressed as the mean ± SEM. Statistical analysis was determined using Student's t-test or one-way ANOVA. All statistical significance (*P < 0.05 and **P < 0.01) was performed using the software SigmaStat (Systat Software, Inc., San Jose, CA, USA).

ACKNOWLEDGEMENTS
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP, NRF-2013R1A1A01069575 and NRF-2016R1A2B3013840) and in part by NRF-2017R1D1B03028379.

CONFLICTS OF INTEREST
The authors have no conflicting interests.

REFERENCES
1. DiLillo DJ, Matsushita T and Tedder TF (2010) B10 cells and regulatory B cells balance immune responses during inflammation, autoimmunity, and cancer. Ann N Y Acad Sci 1183, 38-57
2. Watanabe R, Fujimoto M, Ishiura N et al (2007) CD19 expression in B cells is important for suppression of contact hypersensitivity. Am J Pathol 171, 560-570
3. Yang M, Deng J, Liu Y et al (2012) IL-10-producing regulatory B10 cells ameliorate collagen-induced arthritis via suppressing Th17 cell generation. Am J Pathol 180, 2375-2385
4. Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M and Tedder TF (2008) Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. J Clin Invest 118, 3420-3430
5. Kim HS, Kim AR, Kim DK et al (2015) Interleukin-10-producing CD5+ B cells inhibit mast cells during immunoglobulin E-mediated allergic responses. Sci Signal 8, ra28
6. Mangan NE, Fallon RE, Smith P, van Rooijen N, McKenzie AN and Fallon PG (2004) Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. J Immunol 173, 6346-6356
7. Kim AR, Kim HS, Kim DK et al (2016) Mesenteric IL-10-producing CD5+ regulatory B cells suppress cow's milk casein-induced allergic responses in mice. Sci Rep 6, 19685
8. Zhou J, Min Z, Zhang D, Wang W, Marincola F and Wang X (2014) Enhanced frequency and potential mechanism of B regulatory cells in patients with lung cancer. J Transl Med 12, 304
9. Zhang H, Yue R, Zhao P et al (2017) Proinflammatory follicular helper T cells promote immunoglobulin G secretion, suppress regulatory B cell development, and correlate with worse clinical outcomes in gastric cancer. Tumour Biol 39, 1010428317705747
10. Lu Y, Meng F, Yang Y et al (2017) Significance of B10 cell in patients with thymoma complicated with myasthenia gravis. Oncotarget 8, 7377-7386
11. Sarvari A, Madrigal JA and Saundermont A (2017) B cell regulation in cancer and anti-tumor immunity. Cell Mol Immunol 14, 662-674
12. Yanaba K, Bouaziz JD, Haas KM, Poe JC, Fujimoto M and Tedder TF (2008) A regulatory B cell subset with a unique CD19<sup>+</sup>CD5<sup>+</sup> phenotype controls T cell-dependent inflammatory responses. Immunity 28, 639-650
13. Evans JG, Chavez-Rueda KA, Eddaoudi A et al (2007) Novel suppressive function of transitional 2 B cells in experimental arthritis. J Immunol 178, 7868-7878
14. Xiao S, Brooks CR, Sobel RA and Kuchroo VK (2015) Tim-1 is essential for induction and maintenance of IL-10 in regulatory B cells and their regulation of tissue inflammation. J Immunol 194, 1602-1608
15. Sun J, Wang J, Pefanis E et al (2015) Transcriptomics Identify CD9 as a Marker of Murine IL-10-Competent Regulatory B Cells. Cell Rep 13, 1110-1117
16. Jin G, Hamaguchi Y, Matsushita T et al (2013) B-cell linker protein expression contributes to controlling allergic and autoimmune diseases by mediating IL-10 production in regulatory B cells. J Allergy Clin Immunol 131, 1674-1682
17. Matsushita T, Le Huu D, Kobayashi T et al (2016) A novel splenic B1 regulatory cell subset suppresses allergic disease through phosphatidylinositol 3-kinase-Akt pathway activation. J Allergy Clin Immunol 138, 1170-1182
18. Owen DJ, Ornaghi P, Yang JC et al (2000) The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase gcn5p. Embo J 19, 6141-6149
19. Zeng L and Zhou MM (2002) Bromodomain: an acetyl-lysine binding domain. FEBS Lett 513, 124-128
20. Mele DA, Salmeron A, Ghosh S, Huang HR, Bryant BM and Lora JM (2013) BET bromodomain inhibition suppresses TH17-mediated pathology. J Exp Med 210, 2181-2190
21. Asangani IA, Domneti VL, Wang X et al (2014) Therapeutic targeting of BET bromodomain proteins in castration-resistant prostate cancer. Nature 510, 278-282
22. Garcia PL, Miller AL, Kreizburg KM et al (2016) The BET bromodomain inhibitor JQ1 suppresses growth of pancreatic ductal adenocarcinoma in patient-derived xenograft models. Oncogene 35, 833-845
23. Filippakopoulos P, Qi J, Picard S et al (2010) Selective inhibition of BET bromodomains. Nature 468, 1067-1073
24. Gao F, Yang Y, Wang Z, Gao X and Zheng B (2015) BRAD4 plays a critical role in germline center response by regulating Bcl-6 and NF-κB activation. Cell Immunol 294, 1-8
25. Stanlie A, Yousif AS, Akiyama H, Honjo T and Begum NA (2014) Chromatin reader Brd4 functions in Ig class switching as a repair complex adaptor of nonhomologous
JQ1 suppresses regulatory B cells activation
Min Bum Lee, et al.

end-joining. Mol Cell 55, 97-110
26. Yoshizaki A, Miyagaki T, DiLillo DJ et al (2012) Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions. Nature 491, 264-268
27. Geijtenbeek TB and Gringhuis SI (2009) Signalling through C-type lectin receptors: shaping immune responses. Nat Rev Immunol 9, 465-479
28. Liu Z, Wang P, Chen H et al (2017) Drug Discovery Targeting Bromodomain-Containing Protein 4. J Med Chem 60, 4533-4558
29. Brown JD, Lin CY, Duan Q et al (2014) NF-kB directs dynamic super enhancer formation in inflammation and atherogenesis. Mol Cell 56, 219-231
30. Saraiva M, Cheistensen JR, Tsytyskova AV et al (2005) Identification of a macrophage-specific chromatin signature in the IL-10 locus. J Immunol 175, 1041-1046
31. Hedrich CM, Rauen T, Apostolidis SA et al (2014) Stat3 promotes IL-10 expression in lupus T cells through trans-activation and chromatin remodeling. Proc Natl Acad Sci U S A 111, 13457-13462
32. Choi SY, Lee HH, Lee JH et al (2016) TonEBP suppresses IL-10-mediated immunomodulation. Sci Rep 6, 25726
33. Mauri C and Bosma A (2012) Immune regulatory function of B cells. Annu Rev Immunol 30, 221-241
34. Mizoguchi A, Mizoguchi E, Takedatsu H, Blumberg RS and Bhan AK (2002) Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. Immunity 16, 219-230
35. Fillatreau S, Sweeney CH, McCgeachy MJ, Gray D and Anderton SM (2002) B cells regulate autoimmunity by provision of IL-10. Nat Immunol 3, 944-950
36. Mauri C, Gray D, Mushtaq N and Londei M (2003) Prevention of arthritis by interleukin 10-producing B cells. J Exp Med 197, 489-501
37. Rosser EC and Mauri C (2015) Regulatory B cells: origin, phenotype, and function. Immunity 42, 607-612
38. Balkwill F, Montfort A and Capasso M (2013) B regulatory cells in cancer. Trends Immunol 34, 169-173
39. He Y, Qian H, Liu Y, Duan L, Li Y and Shi G (2014) The roles of regulatory B cells in cancer. J Immunol Res 2014, 215471
40. Zhang Y, Gallastegui N and Rosenblatt JD (2015) Regulatory B cells in anti-tumor immunity. Int Immunol 27, 521-530
41. Carter NA, Rosser EC and Mauri C (2012) Interleukin-10 produced by B cells is crucial for the suppression of Th17/Th1 responses, induction of T regulatory type 1 cells and reduction of collagen-induced arthritis. Arthritis Res Ther 14, R32
42. Nicodeme E, Jeffrey KL, Schaefer U et al (2010) Suppression of inflammation by a synthetic histone mimic. Nature 468, 1119-1123
43. Fu LL, Tian M, Li X et al (2015) Inhibition of BET bromodomains as a therapeutic strategy for cancer drug discovery. Oncotarget 6, 5501-5516
44. Chaidos A, Caputo V and Karadimitris A (2015) Inhibition of bromodomain and extra-terminal proteins (BET) as a potential therapeutic approach in haematological malignancies: emerging preclinical and clinical evidence. Ther Adv Hematol 6, 128-141
45. Barrett E, Brothers S, Wahlestedt C and Beurel E (2014) I-BET151 selectively regulates IL-6 production. Biochim Biophys Acta 1842, 1549-1555
46. Kim HS, Jang JH, Lee MB et al (2016) A novel IL-10-producing innate lymphoid cells (ILC10) in a contact hypersensitivity mouse model. BMB Rep 49, 293-296
47. Kim HS, Lee JH, Han HD et al (2015) Autocrine stimulation of IL-10 is critical to the enrichment of IL-10-producing CD40(high)CD5(+) regulatory B cells in vitro and in vivo. BMB Rep 48, 54-59