BET bromodomain inhibition suppresses T\textsubscript{H}17-mediated pathology

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Interleukin (IL) 17–producing T helper (T\textsubscript{H}17) cells have been selected through evolution for their ability to control fungal and bacterial infections. It is also firmly established that their aberrant generation and activation results in autoimmune conditions. Using a characterized potent and selective small molecule inhibitor, we show that the bromodomain and extra–terminal domain (BET) family of chromatin adaptors plays fundamental and selective roles in human and murine T\textsubscript{H}17 differentiation from naive CD\textsuperscript{4} \textsuperscript{+} T cells, as well as in the activation of previously differentiated T\textsubscript{H}17 cells. We provide evidence that BET controls T\textsubscript{H}17 differentiation in a bromodomain–dependent manner through a mechanism that includes the direct regulation of multiple effector T\textsubscript{H}17–associated cytokines, including IL\textsubscript{17}, IL\textsubscript{21}, and GM-CSF. We also demonstrate that BET family members Brd2 and Brd4 associate with the IL\textsubscript{17} locus in T\textsubscript{H}17 cells, and that this association requires bromodomains. We recapitulate the critical role of BET bromodomains in T\textsubscript{H}17 differentiation in vivo and show that therapeutic dosing of the BET inhibitor is efficacious in mouse models of autoimmunity. Our results identify the BET family of proteins as a fundamental link between chromatin signaling and T\textsubscript{H}17 biology, and support the notion of BET inhibition as a point of therapeutic intervention in autoimmune conditions.

Global analysis of histone modifications in T cells has revealed remarkable differences in the chromatin structure of distinct T helper subsets and some of their signature transcription factors (Wei et al., 2009). Although these and other studies have begun to shed light on the role of chromatin dynamics in the control of immune lineage specification and function (Araki et al., 2009; Ramirez-Carrozzi et al., 2009), how these changes are integrated is still poorly understood. BET polypeptides BRD2, BRD3, BRD4, and BRDT harbor tandem bromodomain motifs that bind acetylated lysine residues in histones, thereby linking changes in chromatin structure with gene transcription. Indeed, BET proteins work as chromatin regulators that recruit transcriptional co-activators, such as P-TEFb, to promote gene transcription in inflammation (Hargreaves et al., 2009; Nicodeme et al., 2010) and cancer (Filippakopoulos et al., 2010; Dawson et al., 2011; Mertz et al., 2011; Zuber et al., 2011).

The dysregulated activation and expansion of CD\textsuperscript{4} \textsuperscript{+} T cells lie at the core of autoimmune disorders. It is now widely recognized that T\textsubscript{H}17 cells, a subset of T helper cells which produce IL-17A, IL-17F, IL-21, IL-22, and GM-CSF, mediate autoimmune conditions including multiple sclerosis, psoriasis, rheumatoid arthritis, and Crohn’s disease, as well as the murine models of these diseases (Bettelli et al., 2006; Korn et al., 2009; Littman and Rudensky, 2010). To differentiate into T\textsubscript{H}17 cells, naive T cells require the combined exposure to TGF-\beta and IL-6, and it is now well established that these two factors work in concert to drive the induction of a transcriptional signature that is largely orchestrated by a group of transcriptional regulators that includes steroid receptor-type nuclear receptors ROR\textsubscript{yt} and ROR\textsubscript{\alpha} (Ivanov et al., 2006; Yang et al., 2008), IRF4 (IFN regulatory factor 4; Brüstle et al., 2007; Ciofani et al., 2012; Glashammer et al., 2012), AP-1 transcription factor Batf (Schraml et al., 2009), the proto-oncogene c-Maf (Bauquet et al., 2009), NF-\kappaB family member c-Rel (Chen et al., 2011; Ruan et al., 2011), and AHR (aryl hydrocarbon receptor; Veldhoen et al., 2008).

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A recent report has revealed a variety of alterations in T cell cytokine production as a consequence of BET bromodomain inhibition (Bandukwala et al., 2012). However, these studies relied on transient pharmacological inhibition shortly after activation, and therefore the role of BET bromodomains during T cell subset differentiation and their potential therapeutic application remain to be defined. In this work, we provide evidence that, through bromodomain function, the BET family of chromatin adaptors play fundamental roles in T_{H}17 cell differentiation in vitro and in vivo, and thus represent a viable therapeutic entry point for a wide range of autoimmune disorders.

RESULTS AND DISCUSSION
BET bromodomains control human T_{H}17 differentiation

Because BET bromodomain inhibition results in the suppression of a subset of NF-κB–dependent genes in murine macrophages (Nicodeme et al., 2010), we hypothesized that BET proteins may also play fundamental roles in other NF-κB–mediated processes, such as T cell activation and differentiation. With that aim, we examined whether BET bromodomain inhibition had any functional impact on the differentiation of naive T cells into any major subsets (T_{H}1, T_{H}2, T_{H}17, and iT reg). To address this question, we used a well characterized potent and selective BET small molecule inhibitor, JQ1 (Filippakopoulos et al., 2010).

Human CD4^{+}CD45RA^{+} naive T cells were purified from peripheral blood and differentiated under standard polarizing conditions in the presence of DMSO or 150 nM JQ1 (inhibitory concentration of 90% response [IC_{90}] in a THP-1 cell-based LPS-induced IL-6 release assay; unpublished data). As a control for specificity we used the inactive enantiomer of JQ1 (JQ1(−)). As shown in Fig. 1, we found a strikingly selective effect on T_{H}17 differentiation. After 6 d of culture, cells treated with DMSO or JQ1(−) exhibited a phenotype consistent with human T_{H}17 cells, as indicated by up-regulation of CCR6, IL-17A, and IL-22 (Fig. 1 A). However, in the presence of JQ1, the number of CCR6^{+} cells was severely reduced, both IL-17A and IL-22 expression were suppressed (Fig. 1 A), and the expression of RORγt (encoded by RORC), RORα, and a subset of T_{H}17 lineage-enriched transcripts were significantly inhibited (Fig. 1 C and Fig. 2 A). Under our differentiating conditions, T_{H}17 cells produce some IL-10, and this was also inhibited by JQ1. BET bromodomain inhibition had no effect on total cell numbers (Fig. 1 D), ruling out the possibility of T_{H}17 suppression as an indirect effect on T cell proliferation. JQ1 had no impact on the differentiation of T_{H}1 (assessed as number of IFN-γ^{+} cells and TBX21 expression), T_{H}2 (IL-4^{+} cells and GATA3 expression), or T reg (FOXP3^{+} cells and FOXP3 expression) cells (Fig. 1, B, E, and F). Thus, BET bromodomains play essential roles in the differentiation of human T_{H}17 cells but not other T cell lineages. The defect in T_{H}17 differentiation uncovered by pharmacological inhibition of BET proteins was recapitulated in siRNA experiments. Because JQ1 binds to the bromodomains of all BET family members, T cells were transfected with combinations of siRNAs targeting BRD2 and BRD4 (BRD3 and BRDT are not expressed in T_{H}17 cells; unpublished data). The combined knockdown of BRD2 and BRD4 for 48 h resulted in significant inhibition of RORα and IL17A expression (unpublished data), confirming the data obtained with JQ1 and further ruling out a nonspecific effect of the small molecule inhibitor. Notably, BRD2 and BRD4 have nonredundant functions in T_{H}17 cells, as knocking down either transcript individually was sufficient to recapitulate the phenotype observed with JQ1 or with double RNA interference (Fig. 2 B). Collectively, these results highlight a critical role for BET bromodomains in promoting human T_{H}17 cell differentiation.

BET bromodomains are necessary for the transcription of multiple T_{H}17 lineage-associated cytokines, including the T_{H}17 autocrine amplification factor IL-21

We consistently observed a complete suppression of IL-21 transcription and protein in JQ1-treated T cells, and in fact as early as 4 h after naïve T cell culture under T_{H}17 differentiating conditions, Il21 was one of the top 20 transcripts most down-regulated upon BET inhibition (unpublished data). IL-21 is required for the generation of T_{H}17 cells as an autocrine factor that induces activation of STAT3 and consequently induction of RORγt (Nurieva et al., 2007; Zhou et al., 2007). Thus, we hypothesized that BET inhibition might control T_{H}17 differentiation, at least in part, by directly regulating IL-21 expression. To address this possibility, we cultured human naïve T cells with recombinant IL-21, and explored the impact of BET bromodomain inhibition on IL-21 mRNA expression. As previously reported (Nurieva et al., 2007; Zhou et al., 2007), exogenous IL-21 was sufficient to induce its own transcription (Fig. 2 C). This response was dependent on functional BET bromodomains, as JQ1 completely suppressed IL21 expression (Fig. 2 C). Moreover, BET bromodomains were also essential for the background IL21 expression observed in the absence of added IL-21 protein (Fig. 2 C, compare lanes 1 and 3). From these observations, we conclude that autocrine IL-21 expression in T cells requires functional BET bromodomains. We next determined the impact of BET inhibition on STAT3 activation and RORγt expression. Early in T_{H}17 differentiation, STAT3 phosphorylation is dominated by the IL-6 present in the culture medium, but over time T cell–derived IL-21 significantly contributes to this process. STAT3 activation downstream of the IL-6 receptor was not affected by BET inhibition, as JQ1 had no impact on STAT3 phosphorylation in naïve cells incubated with IL-6 and TGF-β for 90 min (Fig. 2 D, top). However, BET inhibition resulted in a severe impairment of STAT3 activation and RORγt induction in cells examined after 5 d of culture under T_{H}17-differentiating conditions (Fig. 2, D [bottom] and F [top left]), without affecting STAT3 expression (Fig. 2 E). Addition of exogenous IL-21 almost completely restored STAT3 activation and RORγt expression (Fig. 2, D [bottom] and F [top left]), demonstrating that by directly regulating IL-21 mRNA transcription, BET controls a fundamental amplification loop that contributes to the expression of RORγt and therefore drives optimal T_{H}17 differentiation.
Another critical regulator of T<sub>H</sub>17 differentiation, BATF (Schraml et al., 2009; Ciofani et al., 2012; Glasmacher et al., 2012), was also rescued with exogenous IL-21 (Fig. 2 F). Although the addition of IL-21 circumvented the need of BET to restore RORC and BATF expression, it did not rescue IL-17 production (Fig. 2 F), indicating that BET also directly controls the expression of IL-17. Other critical T<sub>H</sub>17 genes, such as GMCSF, IL22, and IL23R, were suppressed in the presence of JQ1 and were not rescued by addition of exogenous IL-21. BET bromodomains played no role in the control of the expression of the generic proinflammatory cytokine TNF, as JQ1 did not affect its transcript levels (Fig. 2 F). Thus, BET bromodomain inhibition results in the combined and selective suppression of several key T<sub>H</sub>17 lineage-associated genes, such as IL-17, IL-21, GM-CSF, IL-22, and IL-23R. Suppression of IL-21 results in impaired STAT3 activation, and in RORC and BATF expression. We propose that the combination of these effects of BET bromodomain inhibition results in effective suppression of T<sub>H</sub>17 lineage differentiation.

**BET bromodomain inhibition selectively suppresses the production of T<sub>H</sub>17 cytokines in T<sub>H</sub>17 differentiated cells**

A prediction from the observed direct BET requirement for IL17, IL21, and GMCSF transcription is that BET bromodomain inhibition should also impair the production of those cytokines from already differentiated T<sub>H</sub>17 cells. To address this possibility, we differentiated naive T cells under T<sub>H</sub>17 polarizing conditions for 5 d. These newly differentiated T<sub>H</sub>17 cells were then incubated with JQ1 and restimulated for 24 h with anti-CD3 and anti-CD28 in the absence of polarizing cytokines.
Figure 2. BET bromodomain inhibition blocks human T<sub>H17</sub> differentiation by controlling multiple T<sub>H17</sub>-associated genes. (A) Human naive T cells were cultured under T<sub>H17</sub> conditions in the presence of 150 nM JQ1 or DMSO for 48 h and the expression of the indicated genes was investigated by qPCR. Error bars represent standard deviation. Data are representative of two to three independent experiments. Statistical significance was determined by Student’s t test (**, P < 0.01). (B) Human T cells were lentivirally transduced with hairpins targeting BRD2 (red bars) or BRD4 (blue bars) and cultured under T<sub>H17</sub>-polarizing conditions for 6 d. Two individual hairpins per gene were used (sh1 and sh2). Expression of BRD2, BRD4, and RORC was measured by qPCR; NTC, nontargeting control. IL-17A, IL-17F, and IL-21 protein was quantitated by Luminex. Data are representative of two independent experiments. Error bars represent standard deviation. Statistical significance was determined by Student’s t test (*, P < 0.05; **, P < 0.01). (C) Human naive T cells were stimulated with anti-CD3/CD28 for 24 or 48 h in the presence or absence of exogenous human recombinant IL-21 and JQ1 as indicated, and IL21 expression was measured by qPCR. Data are representative of two independent experiments and two independent donors. Error bars represent standard deviation. (D) Human naive T cells were cultured under T<sub>H17</sub>-differentiating conditions for the indicated times in the presence or absence of exogenous human recombinant IL-21 and JQ1 as indicated, and phospho-Stat3 was measured by flow cytometry. Data are representative of two independent experiments and two independent donors. (E and F) Samples from D (5 d, bottom) were analyzed by qPCR for the indicated transcripts. Data are representative of two independent experiments and two independent donors. Error bars...
JQ1 blocked IL-17 production in a dose-dependent manner (not depicted), approaching full suppression at a concentration of 150 nM (Fig. 2 G). In keeping with the notion of a direct role of BET in controlling multiple effector Th17-enriched cytokines, JQ1 also abrogated IL-21 and GM-CSF production (Fig. 2 G). In contrast, the ability of these cells to produce TNF protein was only modestly ameliorated (Fig. 2 H, left) and not reduced at the transcript level (Fig. 2 H, middle). Moreover, another TNF superfamily member, lymphotixin-α, was similarly unaffected (Fig. 2 H, right). IL17, IL21, and GMCSF transcripts were all reduced by JQ1 (unpublished data).

**BET bromodomains control murine Th17 differentiation**

To explore if the role of BET in Th17 differentiation has been conserved throughout evolution, and to enable pharmacological studies in mouse models of autoimmune diseases, we next sought to investigate if BET bromodomains also play critical roles in murine Th17 cell differentiation. Naive mouse T cells were cultured under Th17 polarizing conditions, and their phenotype upon BET inhibition was analyzed after 4 d. Early T cell activation was not affected by BET inhibition, as up-regulation of the early activation marker CD69 was unchanged (Fig. 3 A). Thus, BET bromodomains are dispensable for T cell receptor (TCR) engagement and at least some downstream signaling events. However, Th17 differentiation was largely suppressed, as demonstrated by a significantly reduced number of IL-17A+ cells determined by flow cytometry and reduced IL-17A and IL-22 secretion to the culture medium (Fig. 3 B). This suppression was not generic, as TNF was unchanged upon BET inhibition (Fig. 3 B). To further demonstrate a direct and specific effect of BET on modulating Th17 development, we performed compound titration experiments and monitored IL-17 secretion and cell viability. IL-17 production was suppressed in a dose-dependent manner by JQ1 with an approximate IC50 of 30 nM, whereas cellular viability was unaffected (Fig. 3 C). From these data, we conclude that BET bromodomains are essential for mouse Th17 differentiation.

Given the known roles of BET proteins as transcriptional co-activators, we next investigated whether BET inhibition led to changes in gene expression in T cells during Th17 polarization. Consistent with the failure of naive T cells to differentiate into Th17 cells in the presence of JQ1, we found the transcription of Th17 canonical genes, such as those encoding IL-17A, IL-17F, IL-21, IL-22, IL-23R, RORα, and RORγt, to be significantly reduced by quantitative RT-PCR (qPCR) analysis (unpublished data) after 48 h of polarization. Suppression of proinflammatory gene transcription was selective, as Tnf remained unchanged in the presence of JQ1. Furthermore, JQ1 had no effect on the expression of Il6r (unpublished data), complementing the data reported above (Fig. 2 D, top) for a role of BET in Th17 differentiation independent of early events in IL-6 signaling. These observations suggest that pharmacological blockade of BET bromodomains does not afford general gene suppression but rather a targeted effect on specific transcriptional programs. Of note, BET inhibition did not result in c-Myc transcript reduction (Fig. 2 A and not depicted). This observation suggests a profound cell type–specific function of BET bromodomains, as BET inhibition results in complete suppression of c-Myc transcription in a wide array of cancer cell lines (Dawson et al., 2011; Mertz et al., 2011; Zuber et al., 2011). Therefore, BET proteins can control nonoverlapping transcriptional programs in different cellular contexts. To extend these observations and to gain a comprehensive view of the global transcriptional changes resulting from BET inhibition in Th17 cells, we did full-genome microarray analysis at 48 h of polarization. Strikingly, the transcriptional signature elicited by BET suppression was restricted to only 238 genes (176 down- and 62 up-regulated twofold or more; Fig. 3 D and Table S1). Highly reduced transcripts included those encoding Tg17 signature genes such as Il17a, Il17f, Il23r, and Ccr6 (Fig. 3 D). Other strongly down-regulated transcripts encoded proteins that are not restricted to Th17 cells but have been shown to be relevant for various aspects of Th17 biology, including IL-1R1 (Chung et al., 2009). The expression patterns discussed above were confirmed by qPCR (unpublished data). As previously observed (Nicodeme et al., 2010; Mertz et al., 2011), BET inhibition also resulted in the up-regulation of a small subset of genes (Fig. 3 D and Table S1), suggesting that BET proteins can also act, directly or indirectly, as transcriptional repressors in certain genomic loci and cellular contexts. To further delineate the molecular pathways impinging upon the global transcriptional changes described above, we used the Gene Set Enrichment Analysis (GSEA) algorithm to identify curated gene signatures that significantly coincided with BET inhibition in our transcriptional profiling data. The NF-κB transcription factor target gene set scored as a highly significant overlap with the Th17 expression set (normalized enrichment score = −1.4; P = 0.003; Fig. 3 D, bottom left). This observation is consistent with published data demonstrating a role of BET proteins in mediating NF-κB–dependent gene transcription in other cellular contexts (Hargreaves et al., 2009; Nicodeme et al., 2010). Collectively, the results reported here demonstrate that BET proteins, through their bromodomain function, play a critical role in orchestrating the transcriptional program that drives T cell differentiation into the Th17 subset, possibly by modulating the transcriptional state of NF-κB–dependent genes. In keeping with our data from human T cells, we also observed that BET bromodomain inhibition results in a significant and dose-dependent attenuation of
used the mouse system as a source of large number of cells to perform chromatin immunoprecipitation (ChIP) studies under TH17 polarizing conditions. After 20 h of culture, both Brd2 and Brd4 significantly associated with chromatin at the Il17a control region CNS2 (essential for Il17a transcription; Akimzhanov et al., 2007) in TH17 but not TH1 or TH2 cells, and this binding was prevented by JQ1, significantly for Brd4 (Fig. 3 F). JQ1 had no impact on H3K4me3 or acetylation on CNS2 (Fig. 3 F), suggesting that the reduced association of BET at this site was not an indirect consequence of altered chromatin structure. Furthermore, the fact that H4Ac4 levels were unaffected (Fig. 3 F) suggests that the reduced binding of BET at CNS2 is the result of JQ1-mediated release from chromatin, and not simply an absence of the bromodomain.

IL-17 production in fully differentiated mouse T\textsubscript{H}17 cells (Fig. 3 E). Although our data clearly demonstrate that BET bromodomains selectively control T\textsubscript{H}17 differentiation, it is possible that they might modulate some biological functions of other T cell lineages, as BET inhibition results in the down-regulation of multiple genes in T\textsubscript{H}0 cells (unpublished data).

BET members Brd2 and Brd4 directly bind to the \textit{Il17} genomic locus in a bromodomain-dependent manner

Our observation of a requirement of functional BET bromodomains for IL-17 mRNA generation (Fig. 2, A and F) prompted us to mechanistically elucidate whether the two BET family members expressed in T\textsubscript{H}17 cells, Brd2 and Brd4, associated with chromatin at the \textit{Il17} locus. With that aim, we used the mouse system as a source of large number of cells to perform chromatin immunoprecipitation (ChIP) studies under T\textsubscript{H}17 polarizing conditions. After 20 h of culture, both Brd2 and Brd4 significantly associated with chromatin at the \textit{Il17a} control region CNS2 (essential for \textit{Il17a} transcription; Akimzhanov et al., 2007) in T\textsubscript{H}17 but not T\textsubscript{H}1 or T\textsubscript{H}2 cells, and this binding was prevented by JQ1, significantly for Brd4 (Fig. 3 F). JQ1 had no impact on H3K4me3 or acetylation on CNS2 (Fig. 3 F), suggesting that the reduced association of BET at CNS2 is the result of JQ1-mediated release from chromatin, and not simply an absence of the bromodomain.

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circulating IL-17 in JQ1-treated animals (Fig. 3 H, left). It has been established that in this model, the duodenal T H17 cells acquire a regulatory phenotype that includes the coexpression of \( \text{Il17} \) and \( \text{Il10} \) (Esplugues et al., 2011). Consistent with those observations, we also found a significant reduction of circulating IL-10 in the plasma of these mice upon BET inhibition (Fig. 3 H, right). It is possible that this reduction in IL-10 production is a direct transcriptional effect of BET in activated effector cells (Fig. 3 D), or a consequence of reducing the numbers of TH17 (regulatory TH17) cells generated in this model. Collectively, these data demonstrate a critical role for the BET bromodomains in the generation of TH17 cells in vivo.

**Inhibition of BET bromodomains protects mice from autoimmunity**

We next investigated if T117 suppression by BET inhibition could be recapitulated in vivo. We used a well established model where, upon two sequential injections of anti-CD3 antibody (as a way to strongly engage the TCR), an immunoregulatory environment is generated that promotes T117 differentiation (Esplugues et al., 2011). Consistent with previous reports demonstrating migration of T117 cells into the small intestine in this model, we observed a population of IL-17–producing T cells in the duodenum. Treatment of mice with the BET inhibitor resulted in a marked suppression of this duodenal T117 population (Fig. 3 G). In accordance with these observations, we also observed a significant reduction in the levels of circulating IL-17 in JQ1–treated animals (Fig. 3 H, left). It has been established that in this model, the duodenal T117 cells acquire a regulatory phenotype that includes the coexpression of \( \text{Il17} \) and \( \text{Il10} \) (Esplugues et al., 2011). Consistent with those observations, we also found a significant reduction of circulating IL-10 in the plasma of these mice upon BET inhibition (Fig. 3 H, right). It is possible that this reduction in IL-10 production is a direct transcriptional effect of BET in activated effector cells (Fig. 3 D), or a consequence of reducing the numbers of T117 (regulatory T117) cells generated in this model. Collectively, these data demonstrate a critical role for the BET bromodomains in the generation of T117 cells in vivo.

**Inhibition of BET bromodomains protects mice from autoimmune**

The fundamental involvement of T117 cells in the pathogenesis of autoimmunity is firmly established. Because our data demonstrate that BET bromodomains are critical in the molecular target. Thus, BET bromodomain inhibition results in impaired binding of Brd2 and Brd4 to the \( \text{Il17a} \) gene and its suppressed transcription.
of TH17 cells once they are fully differentiated. Because BET bromodomains play a pivotal role in the development and maintenance of CIA.

In a separate set of experiments, mice were immunized with a myelin oligodendrocyte glycoprotein (MOG) peptide, and when the initial signs of disease became evident (clinical score = 1) animals were treated with vehicle, JQ1, or dexamethasone (as a generic antiinflammatory control). JQ1-treated animals were significantly protected from experimental arthritis, both macroscopically (Fig. 4 A) and histologically (Fig. 4 B). Importantly, no obvious adverse events were observed in any animal during the treatment period, as determined by monitoring body weight over the course of the experiment (unpublished data). From these data, we conclude that BET bromodomains play a pivotal role in the development and maintenance of CIA.

In summary, we have shown that BET proteins, through their bromodomain motifs, play critical roles in the control of TH17 cells in this model.
Cell viability. Cell viability was assessed using Cell Titer Glo which determines the number of live cells based on quantification of ATP present (G7572; Promega). Live cell numbers were determined by trypan blue staining followed by analysis using Countess automated cell counter (Invitrogen).

shRNAs and lentivirus transduction of human primary T cells. Target sequences for human BRD2 and BRD4 were obtained from Cellecta. Sense and antisense oligonucleotides (66 nt) containing sense, loop, and antisense sequences were obtained with 5′ phosphorylation from Integrated DNA Technologies. Oligonucleotides were annealed and ligated into a lentiviral shRNA vector based on pLKO.1 obtained from Cellecta. In this vector, shRNA expression is driven by the U6 promoter, and both a fluorescence marker and puromycin acetyltransferase (separated by the T2A sequence) are driven by the UbiC promoter. Lentiviral production was performed according to publicly available protocols (http://www.broadinstitute.org/rnaist/public/resources/protocols). Naïve T cells were differentiated under Th17 polarizing conditions for 48 h. Total

RNA was prepared using an RNeasy kit with on-column DNase digestion proofed by WuXi’s IACUC. C57BL/6 mice were randomized into four groups and immunized subcutaneously via the tail vein using purified chicken type II collagen emulsified at a 1:1 ratio (vol/vol) in CFA, and then boosted 3 wk later using chicken type II collagen emulsified at a 1:1 ratio (vol/vol) in IFA. Approximately 2 wk after the boost, animals began to show disease signs and treatment was initiated (day 0), and it continued for 14 d.

EAE. EAE studies were performed at WuXi App Tech Ltd and were approved by WuXi’s IACUC. C57BL/6 mice were randomized into four groups and immunized subcutaneously at the base of the tail with 100 µg/mouse MOG peptide (MOG35-55, MEVGWYRSPFSRVVHLYRNGK) emulsified in CFA containing 5 mg/ml Mycobacterium tuberculosis H37Ra. On days 0 and 2 of immunization, mice were given an i.p. injection of 0.5–1 µg pertussis toxin. Animals were scored daily for clinical signs of EAE using the following criteria: 0, normal; 1, limp tail or hind limb weakness but not both; 2, limp tail and hind limb weakness; 3, partial hind limb paralysis; 4, complete hind limb paralysis; 5, moribund state or death. Treatment was initiated ~2 wk after immunization, when the mean of clinical scores reached 1 and lasted for the remainder of the study. Mice were administered JQ1 at 30 mg/kg, i.p. twice daily, DMSO (vehicle) i.p. twice daily, and dexamethasone at 1 mg/kg orally once a day.

Chemical compound synthesis. JQ1 was synthesized according to published methods (Filippakopoulos, et al., 2010).

Online supplemental material. Table S1 shows the effect of BET bromodomain inhibition on gene expression in Th17 cells. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20130376/DC1.

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The authors are employees of Constellation Pharmaceuticals and hold stock options in Constellation Pharmaceuticals. The authors declare no further conflicts of interest.

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REFERENCES


Table S1. Effect of BET bromodomains inhibition on gene expression in TH17 cells

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Table S1. Effect of BET bromodomains inhibition on gene expression in Tp17 cells (Continued)

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Mouse naive T cells were cultured under Th17 conditions for 48 h in the presence of 150 nM JQ1 or DMSO control, and global expression analysis was carried out on the Affymetrix exon array platform. Genes up- or down-regulated at least 1.5-fold (P < 0.1) are shown.