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BET bromodomain inhibition as a novel strategy for reactivation of HIV-1

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ABSTRACT

The persistence of latent HIV-1 remains a major challenge in therapeutic efforts to eradicate infection. We report the capacity for HIV reactivation by a selective small molecule inhibitor of BET family bromodomains, JQ1, a promising therapeutic agent with antioncogenic properties. JQ1 reactivated HIV transcription in models of latent T cell infection and latent monocyte infection. We also tested the effect of exposure to JQ1 to allow recovery of replication-competent HIV from pools of resting CD4$^+$ T cells isolated from HIV-infected, ART-treated patients. In one of three patients, JQ1 allowed recovery of virus at a frequency above unstimulated conditions. JQ1 potently suppressed T cell proliferation with minimal cytotoxic effect. Transcriptional profiling of T cells with JQ1 showed potent down-regulation of T cell activation genes, including CD3, CD28, and CXCR4, similar to HDAC inhibitors, but JQ1 also showed potent up-regulation of chromatin modification genes, including SIRT1, HDAC6, and multiple lysine demethylases (KDMs). Thus, JQ1 reactivates HIV-1 while suppressing T cell activation genes and up-regulating histone modification genes predicted to favor increased Tat activity. Thus, JQ1 may be useful in studies of potentially novel mechanisms for transcriptional control as well as in translational efforts to identify therapeutic molecules to achieve viral eradication. *J. Leukoc. Biol.* 92: 000–000; 2012.

Introduction

Whereas ART has profoundly improved mortality in HIV-infected individuals, low levels of viral expression persist, necessitating lifelong treatment [1–8]. In landmark studies [8–10], CD4 T cells obtained from patients on combination ART were shown to carry detectable proviral DNA that were capable of cellular activation in vitro, suggesting the presence of latent viral reservoirs. The persistence of viral reservoirs imposes the requirement for lifelong ART, allowing viral rebound upon treatment interruption. Therefore, strategies for elimination of viral reservoirs are of great importance in efforts to cure HIV-1 infection.

HIV-1 proviral latency represents an ensemble of proviral molecular states that includes a majority of defective or actively suppressed proviral DNA, as well as a much smaller population (~1% of proviral integrants) that is inducible with cellular activation [11]. Furthermore, latently infected resting CD4 T cells containing replication-competent virus are undetectable by common clinical tests (reviewed in refs. [12, 13]). Studies in cell lines and in vitro primary cell systems have shown that latent provirus is often associated with HDAC occupancy [14] and heterochromatin modifications of the HIV promoter (LTR) [13, 15], thought to limit transcription initiation and thus, maintain viral latency [13, 16]. Early but unsuccessful attempts at purging the latent pool of infected cells were performed using IL-2 and other mitogenic stimuli that mimicked T cell activation [17, 18]. Hexamethylenebisacetamide, a hybrid bipolar compound, had also been shown to induce HIV-1 expression in latently infected resting memory CD4 T cells obtained from aviremic patients treated with ART [19]. Other more recent approaches have been largely based on HDAC inhibition. Valproic acid [20] appeared to have some effect on the frequency of resting cell infection in a small initial study, but later studies showed that depletion of resting cell infection was infrequently seen and/or limited [21–23]. Currently, other compounds, particularly HDAC

Abbreviations: 7AAD=7-amino actinomycin D, ART=antiretroviral therapy, BET=bromodomain and extra terminal domain, BRD2/3/4=bromodomain 2/3/4, CDK9=cyclin-dependent kinase 9, CycT1/CCNT1=cyclin T1, DAVID=Database for Annotation, Visualization and Integrated Discovery, GO=gene ontology, HDAC=histone deacetylases, HEXIM1=hexamethylene bisacetamide-inducible protein 1, KDM=histone lysine demethylase, P-TEFb=positive transcription elongation factor b, qRT-PCR=quantitative RT-PCR, RNAP II=RNA polymerase II, SAHA=suberoylanilide hydroxamic acid, SIRT1=sirtuin 1, TAR=Tat responsive region, TSA=trichostatin-A

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

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modulators, are in various stages of development [24]. Among the first in vitro cell lines used to study HIV latency were the Ach2 T cell line and the U1 promonocytic cell line, both characterized by low but constitutive expression of HIV-1 that can be potently up-regulated upon treatment with cytokines and/or mitogens [25–27]. Notably, the proviruses in these cell lines contain mutations in the HIV-encoded Tat protein (U1) and in the RNA-binding element TAR (Ach2). A full-genome latent T cell line, J-Lat, with functional Tat and GFP replacing *nef* has also been used as a model for latency [28, 29].

HIV Tat has the capacity to recruit the cellular pause relief factor, P-TEFb, to the HIV LTR, thereby enhancing processive RNAP II transcription. P-TEFb, a heterodimer composed of CycT1 and Cdk9, increases RNAP II activity by hyperphosphorylating the carboxyl terminal domain of RNAP II. Fluctuation in Tat levels below a critical threshold has been proposed to be important in the establishment of latency in CD4 T cells [30]. Thus, viral Tat and cellular P-TEFb levels are key regulators that operate in the context of multiple host factors to influence latency and viral reactivation.

The small molecule JQ1, developed by J.B. and colleagues, is a cell-permeable compound with potent cancer suppressor activity. JQ1 recognizes sites that bind acetylated lysine residues, i.e., bromodomains, with highest specificity for BRD4 and lower specificity for Brd2 and Brd3 [31]. As the host cofactor BRD4 interacts with both subunits of P-TEFb, it may compete with Tat for binding to P-TEFb at the HIV promoter [32]. Furthermore, as JQ1 inhibits BRD4 activity, and BRD4 interacts with P-TEFb to modulate HIV transcription, we hypothesized that JQ1 might influence HIV transcription in latency through this pathway. Therefore, we were interested in evaluating a potential role for JQ1 in HIV transcription and viral reactivation.

MATERIALS AND METHODS

Cell culture

Ach2, U1, and J-Lat (full-length clone 10.6) cell lines were obtained from U.S. National Institutes of Health AIDS Research and Reagent Program. Cells were cultured in RPMI, supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin-streptomycin (Life Technologies, Grand Island, NY, USA; complete media). Primary CD4 cells were obtained from Source Leukocytes (New York Biologics, Southampton, NY, USA), isolated using Histopaque-1077 (Sigma-Aldrich, St. Louis, MO, USA) and a Miltenyi CD4 T cell isolation kit II (Miltenyi Biotec, Auburn, CA, USA), and cultured in complete media supplemented with IL-2.

Cell stimulations

TNF-α (Sigma-Aldrich) was used at a concentration of 0.5 ng/ml. PHA (Sigma-Aldrich) was used at a concentration of 1 μg/ml. IL-2 (R&D Systems, Minneapolis, MN, USA) was used at a concentration of 10 ng/ml. Human αCD3 and αCD28 (BD Biosciences, San Jose, CA, USA) were used at a concentration of 0.1 μg/ml and 1 μg/ml, respectively. PMA (Calbiochem/EMB Chemicals, Merck, Darmstadt, Germany) was used at 1 nM. JQ1S and JQ1R were provided by J.B. (Harvard Medical School, Boston, MA, USA).

qRT-PCR

qRT-PCR was run on RNA isolated from cells using TRIzol (Life Technologies) or the RNeasy kit (Qiagen, Valencia, CA, USA) with the respective, suggested protocols. cDNA for use with TaqMan probes was synthesized using a high-capacity cDNA synthesis kit (Life Technologies). The HIV primer probes were custom-made [33] and ordered from Applied Biosystems (Foster City, CA, USA; forward-TACTGACGCTCTCGCACC, reverse-TCTCGACGCAAGGACTCG, FAM-CTCTCTCCTTCTAGCCTC). Real-time amplification results were all normalized to an endogenous control human GAPDH FAM probe (Life Technologies). Samples were run on an ABI Prism 7000 sequence detection system and analyzed using ABI Prism 7000 sequence detection (Applied Biosystems). The relative quantities were calculated using the ΔΔcomparative threshold method.

Flow cytometry analysis

After compound treatments, GFP, 7AAD, Annexin V, and CD4 were measured using an LSRII (Becton Dickinson, San Diego, CA, USA). Data were analyzed using FACSDiva (Becton Dickinson) and FlowJo 9.4 (Tree Star, Ashland, OR, USA). Anti-CD4-FITC was from eBioscience (San Diego, CA, USA). Annexin V and 7AAD reagents were purchased from Invitrogen (Carlsbad, CA, USA). Results shown are representative data of experiments repeated five times. 7AAD and Annexin V expression was measured on gated cells, based on forward/side-scatter gating and CD4 surface staining to obtain a homogeneous population. Analysis was conducted on 50,000 cells/condition.

Viral infections

Ach2 cells were used to obtain viral supernatants to use for infection of primary cells. PMA was used to stimulate HIV activation of Ach2 cells for 24 h. Cells were concentrated in fresh complete medium without stimulant and viral supernatants collected after 48 h using centrifugation and filtration and used for subsequent infections in vitro.

Cell proliferation assays (MTT)

Cell proliferation assays were conducted using the Cell Proliferation Kit I (MTT; Roche Applied Science, Indianapolis, IN, USA). J-Lat and primary CD4 cells were plated in suspension culture with mock treatment, 500 nM, 50 nM, and 5 nM JQ1S. After 24 h treatment, the MTT assay was conducted as specified by the manufacturer's protocol. Statistics were done using single-factor ANOVA with significance at $P < 0.05$.

Limiting dilution outgrowth assays of resting $CD4^+$ T cells from HIV-1-positive patients

This method has been described in detail [23]. In summary, to measure the frequency of infected units/billion resting $CD4^+$ T cells for a patient, cells were maximally stimulated with 1 μg/ml PHA-L (Remel Products, Thermo Fisher Scientific, Lenexa, KS, USA) and a fivefold excess of allogeneic, irradiated PBMCs from a seronegative donor or with 0.1–1 μM JQ1 or 20 U IL-2 alone for 24 h, then washed, and placed in limiting dilution cultures. PHA-stimulated, CD8-depleted PBMCs from selected seronegative donors were added to cultures to permit amplification of expressed virus, as described previously [23]. All cultures were maintained in IMDM with 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 20 U IL-2. Cultures were split, and fresh medium was added as described previously [34] to ensure optimal growth conditions.

Transcriptional microarray profiling

J-Lat cells were stimulated with αCD3 and αCD28, 1000 nM JQ1S, or 100 nM JQ1S for 24 h. RNA was isolated using RNeasy. Genome-wide microarrays were run on human gene ST 1.0 arrays (Affymetrix, Santa Clara, CA, USA). Gene-level expression measurements were generated using the robust multichip average algorithm and a library file that collapses gene expression measurements to ~20,000 unique Entrez Gene IDs. ANOVA was used to identify genes whose expression varied between conditions with an ANOVA significance of $P < 1E\text{-}5$. The cutoffs were chosen arbitrarily to produce heatmaps with ~300 genes. DAVID [35, 36] analysis, using stan-

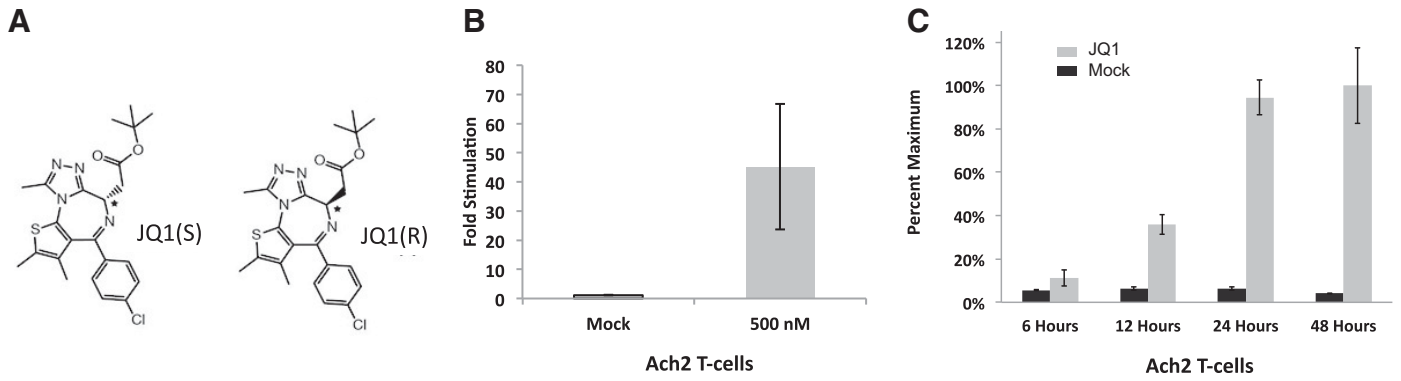

Figure 1. JQ1 reactivates HIV transcription in Ach2 T cells and U1 promonocytes. (A) Two enantiomers of JQ1 have been described: the S and R forms [31]. Asterisks indicate the stereocenter at C6. (B) Ach2 T cells were treated with JQ1S at 500 nM for 24 h, followed by measurement of viral transcription with qRT-PCR. In all cases, the specific inhibitor JQ1 (S, 500 nM) but not the stereoisomer control JQ1 (R, 500 nM) dose-responsively reactivated HIV-1 RNA transcription (B and Supplemental Fig. 1 [37]). (C) Representative time course experiment indicating that maximal JQ1 stimulation (500 nM) of Ach2 cells occurred within 24 h. Similar results were seen with U1 cells (data not shown). All experiments were repeated three times. Error bars on graphs show SD between samples. All differences were found to be significant using a Student's *t* test to $P < 0.05$ for bar graphs.

dard parameters and medium stringency, was conducted to identify clusters and GO categories.

RESULTS AND DISCUSSION

JQ1 reactivates latent HIV transcription in Ach2 T cells and U1 promonocytes

JQ1 has been described previously to bind competitively to acetyl-lysine recognition motifs or bromodomains, specifically BRD4. As BRD4 influences HIV expression, we were interested in evaluating a potential role for JQ1 in HIV transcription. Two enantiomers of JQ1 have been described: the S and R forms, shown in **Fig. 1A** and ref. [31]. We tested the ability of JQ1S to reactivate latent virus in the T cell line, Ach2, and U1 promonocytic cell line. As shown in Fig. 1, the specific inhibitor JQ1 (S, 500 nM), but not a stereoisomer control JQ1 (R, 500 nM; data not shown and Supplemental Fig. 1 [37]), potently reactivated HIV-1 RNA transcription in the latently infected T cell line (Ach2, Fig. 1B). Notably, maximal activation appeared to occur within 24 h (Fig. 1C). Similar results were seen in U1 cells and three independent latent T cell clones (data not shown and Supplemental Fig. 1).

JQ1 reactivates latent HIV in the J-Lat T cell line

Although useful for transcriptional studies, the proviruses in U1 and Ach2 cell lines contain mutations in the Tat protein (U1) and in the Tat RNA element TAR (Ach2). As these lines have Tat–TAR mutations, their relevance to latency may be limited. A full-genome latent T cell line, J-Lat, with functional Tat and GFP replacing *nef* has also been used as a model for latency [28, 29, 38]. Therefore, we evaluated the ability for JQ1S to reactivate HIV from J-Lat cells (Clone 10.6). We treated J-Lat cells for 24 h with reagents known to reverse latency in this model or 500 nM JQ1S and then examined the cells using flow cytometry. As shown in **Fig. 2**, we observed an increase in GFP expression when the cells were stimulated

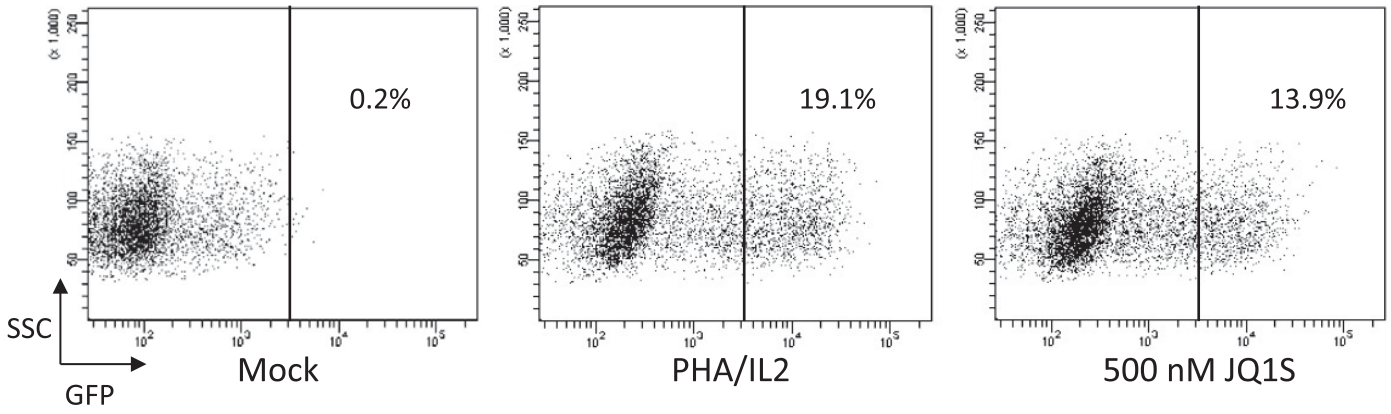

Figure 2. JQ1 reactivates HIV transcription in J-Lat T cell line. The latent J-Lat 10.6 cell line was treated with mock (complete media), 500 nM JQ1S, or PHA/IL-2 as a reference control for 24 h, followed by analysis using flow cytometry. As indicated, stimulation with PHA and IL-2 or JQ1S resulted in an increase in GFP expression. Flow data are representative results of $n = 5$ experiments. SSC, Side-scatter.

with PHA/IL-2 (or PMA; data not shown), as well as when cells were exposed to JQ1S compared with mock stimulation (Fig. 2).

JQ1 effects in stimulated and resting CD4$^+$ T cells

To determine whether JQ1 activation was limited to unstimulated conditions or whether JQ1-mediated activation might synergize with other viral inducers, we activated Ach2 T cells with TNF and measured viral transcription 24 h after compound addition using qRT-PCR. Viral transcription was augmented by the addition of JQ1S but not JQ1R in Ach2 T cells (**Fig. 3A**). Similar results were seen with U1 promonocytes (data not shown).

To determine whether JQ1 influences HIV-1 transcription in the context of acute T cell infection, we stimulated purified primary CD4 T cells with the mitogen cocktail PHA/IL-2 for 3 days and then infected with HIV-1. After 3 days of infection, cells were exposed to JQ1 or control for 24 h. As shown in Fig. 3B, we observed a potent stimulation of HIV-1 transcription after 24 h of JQ1 treatment compared with mock treatment, similar to that seen in the primed Ach2 and U1 cells, indicating that JQ1 potently activates HIV-1 in acutely infected primary CD4 T cells.

We then tested the effect of exposure to JQ1 on recovery of replication-competent HIV from pools of resting CD4$^+$ T cells isolated from HIV-infected, ART-treated patients. In one of three patients (Fig. 3C), exposure to 1 μM JQ1 allowed recovery of virus at a frequency above that seen in unstimulated culture conditions (IL-2, 20 U/ml).

JQ1 has minimal cytotoxicity but potently suppresses proliferation of CD4 T cells

As JQ1S is a BRD4 antagonist and as BRD4 allows for progression of cells from the G1- to S-phase of the cell cycle [39], we were interested in whether JQ1S influences cytotoxicity and/or cellular proliferation using primary CD4 T cells. To measure cytotoxicity, purified CD4$^+$ T cells (**Fig. 4A**) were treated with 500 nM JQ1 for 24 h and analyzed by flow cytometry for the apoptotic/necrotic markers 7AAD and Annexin V (Fig. 4B). Based on quadrant analysis, JQ1 negligibly increased early apoptosis (7AAD−Annexin V+ cells) and modestly increased late apoptosis/necrosis (7AAD+Annexin V+ cells) compared with a reference control staurosporine, as shown in Fig. 4C. To evaluate proliferative capacity, cells were treated with increasing concentrations of JQ1S (5 nM, 50 nM, and 500 nM), and then a MTT assay was performed. Similar to the HDAC inhibitors TSA and SAHA, JQ1 inhibited cell proliferation dose-responsively (Fig. 4D). Notably, the affect on cell proliferation by JQ1 was below that seen with TSA, where 5 nM was a 50% inhibitory dose [40]. Our results were also consistent with JQ1 up-regulation of cell-cycle arrest genes [31, 41].

JQ1 up-regulates chromatin modification genes

To characterize global gene expression in response to JQ1 treatment, J-Lat 10.6 T cells were transcriptionally profiled by microarray after 24 h treatment with JQ1 (100 nM or 1 μM) or mitogenic human αCD3/αCD28, which induces T cell activation and activates HIV expression (**Fig. 5**, and data not shown). There was a global distinction in gene regulation by JQ1, distinct from αCD3/αCD28. To identify gene categories influenced by JQ1, we conducted gene set enrichment analysis using Expression Analysis Systematic Explorer and DAVID software [35, 36] and compared categories of genes induced and suppressed by JQ1 with genes regulated by αCD3/αCD28 stimulation. The top category induced by JQ1 was the GO category chromatin organization and included histone demethylases KDM4A, KDM5A, KDM5B, and KDM6A and histone acetyltransferases P300/CBP-associated factor/K(lysine) acetyltransferase 2B, monocytic and myeloid leukemia 3 (Fig. 5, Cluster C).

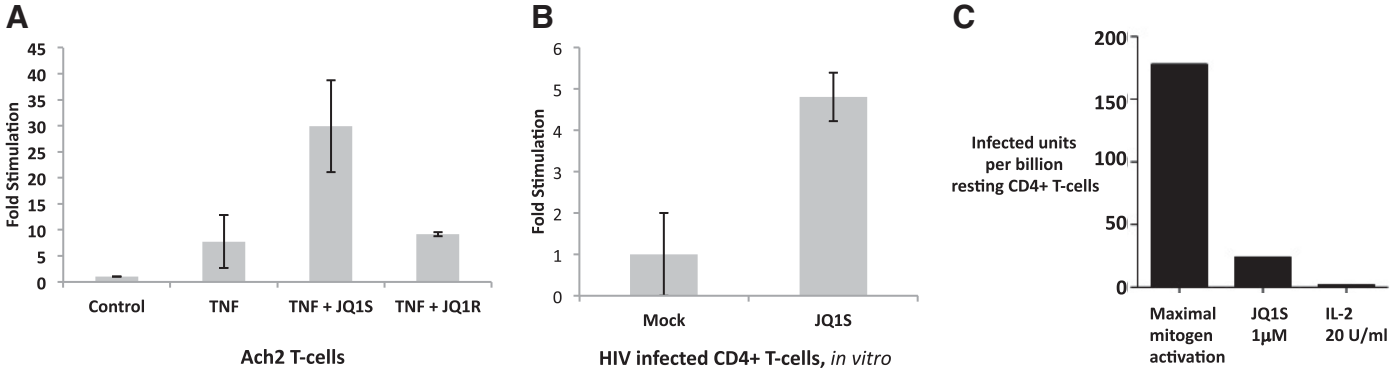

Figure 3. JQ1 effects in vitro and ex vivo using stimulated and resting CD4$^+$ T cells. (A) Ach2 T cells were treated with 0.5 ng/ml TNF-α for 1 h followed by treatment with JQ1S at 500 nM for 24 h. HIV transcription was augmented when JQ1S was added compared with TNF treatment alone (similar results were observed with PMA; data not shown). (B) Primary CD4 T cells were treated with PHA/IL-2 and virus for 3 days before being stimulated with JQ1S for an additional 24 h. JQ1 reproducibly augmented expression of HIV transcription as indicated. All in vitro experiments were repeated at least three times. Error bars on graphs show SD between samples. All differences were found to be significant using a Student's t test to $P < 0.05$ for bar graphs. (C) JQ1-mediated recovery of virus from pools of resting CD4$^+$ T cells isolated from HIV-infected, ART-treated patients. In one of three patients, exposure to 1 μM JQ1 allowed recovery of virus above that seen in unstimulated culture conditions (IL-2, 20 U/ml).

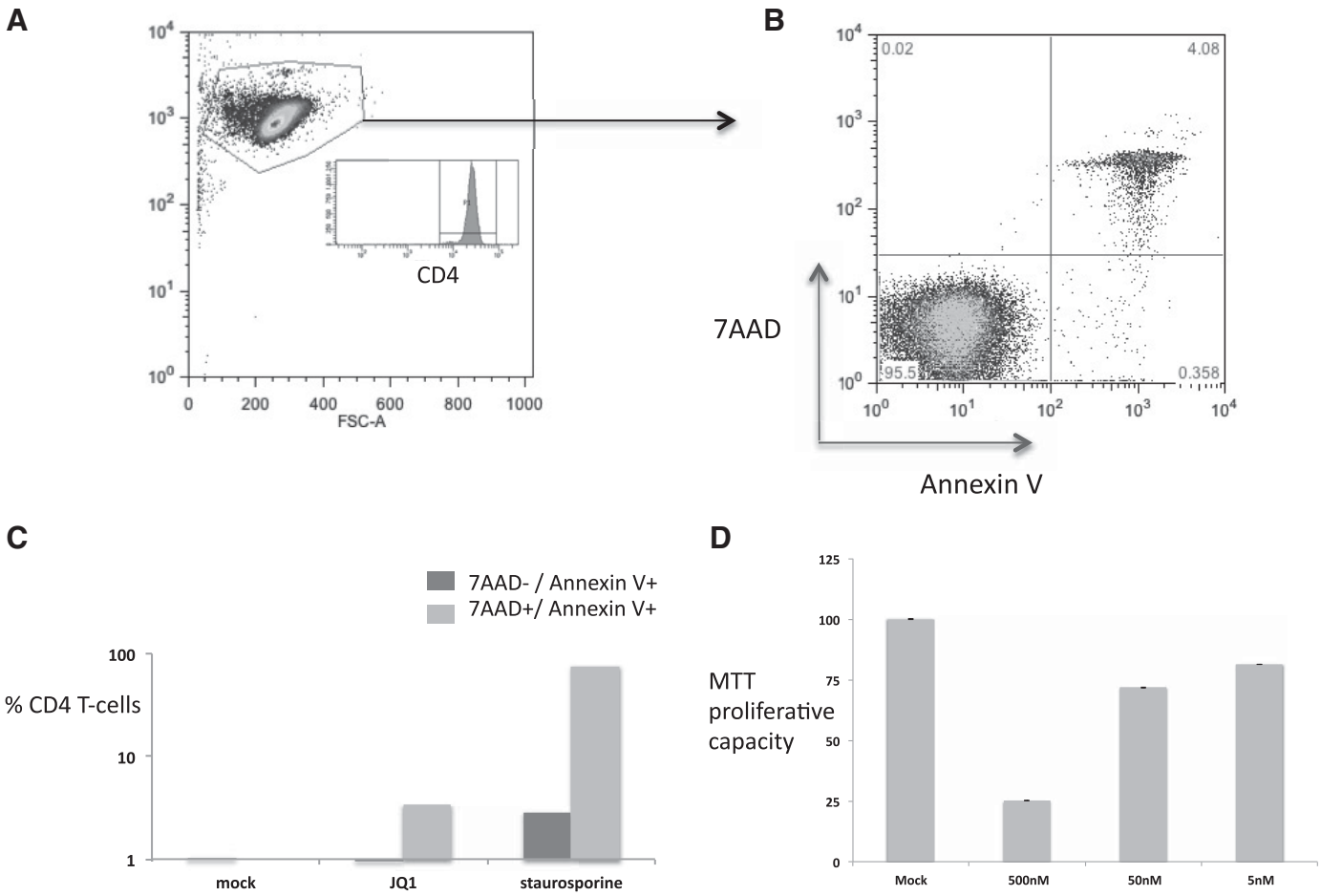

Figure 4. Influence of JQ1 on CD4$^+$ T cell cytotoxicity and proliferation. Cytoxicity was measured by flow cytometry analysis of 7AAD and Annexin V staining after primary T cell treatment with mock (complete media) or 500 nM JQ1S for 24 h. For proliferation measurements based on MTT assay, cells were mock-treated or treated with varying concentrations of JQ1S (500 nM, 50 nM, 5 nM). (A) Flow cytometric gating for uniform cells indicate that nearly all cells purified from whole blood by magnetic beads were CD4-positive. FSC-A, Forward-scatter-area. (B) Cellular gating strategy to identify apoptotic and necrotic cells. (C) Based on quadrant analysis, JQ1 negligibly increased early apoptosis (7AAD−Annexin V+ cells) and modestly increased late apoptosis/necrosis (7AAD+Annexin V+ cells) compared with a reference control staurosporine. (D) Primary T cell proliferation was dose-responsively suppressed by JQ1, consistent with previous reports of JQ1 in other cell types [31, 41]. For these experiments, $n = 5$; error bars show SD between samples, and all differences were found to be significant using ANOVA to $P < 0.05$.

JQ1 down-regulates T cell activation genes

The top category of repressed genes by JQ1 was the GO category lymphocyte activation and included CD2, CD3D, CD3E, CD3G, CXCR4, CD28, and CD40 ligand (Fig. 5, Cluster D). This repression was essentially opposite the expression profile stimulated by αCD3/αCD28 treatment, wherein the top-induced GO category was leukocyte activation (Fig. 5, Cluster A). The top-repressed GO category with αCD3/αCD28 stimulation was membrane-enclosed lumen (Fig. 5, Cluster B). The down-regulation of T cell activation genes observed with JQ1 was far more pronounced than other HIV reactivation compounds that modulate T cell genes, such as the HDAC inhibitors TSA [40] and SAHA [42]. Thus, these reagents may differ from JQ1 in underlying mechanisms of viral reactivation.

JQ1 regulates genes associated with HIV transcription

To determine whether JQ1 influences genes associated with HIV transcriptional control, we compiled a gene list based on literature review of HIV regulators, with an emphasis on regulators of Tat activity [43]. As shown in Fig. 5 (Cluster E), these genes were differentially regulated in response to JQ1 compared with αCD3/αCD28 stimulation. Notably, we observed that the P-TEFb component CycT1/CCNT1, to which Tat binds cooperatively, was induced with JQ1 but not by αCD3/αCD28. However, the CDK9 component of P-TEFb was not appreciably affected by either treatment, but a target of CDK9, the negative elongation factor [44–46] subunits A and C, was down-regulated selectively by JQ1. The methyltransferases, protein arginine *N*-methyltransferase 6 and SETDBI/KMT1E, which negatively affect Tat [47, 48], were down-regulated by

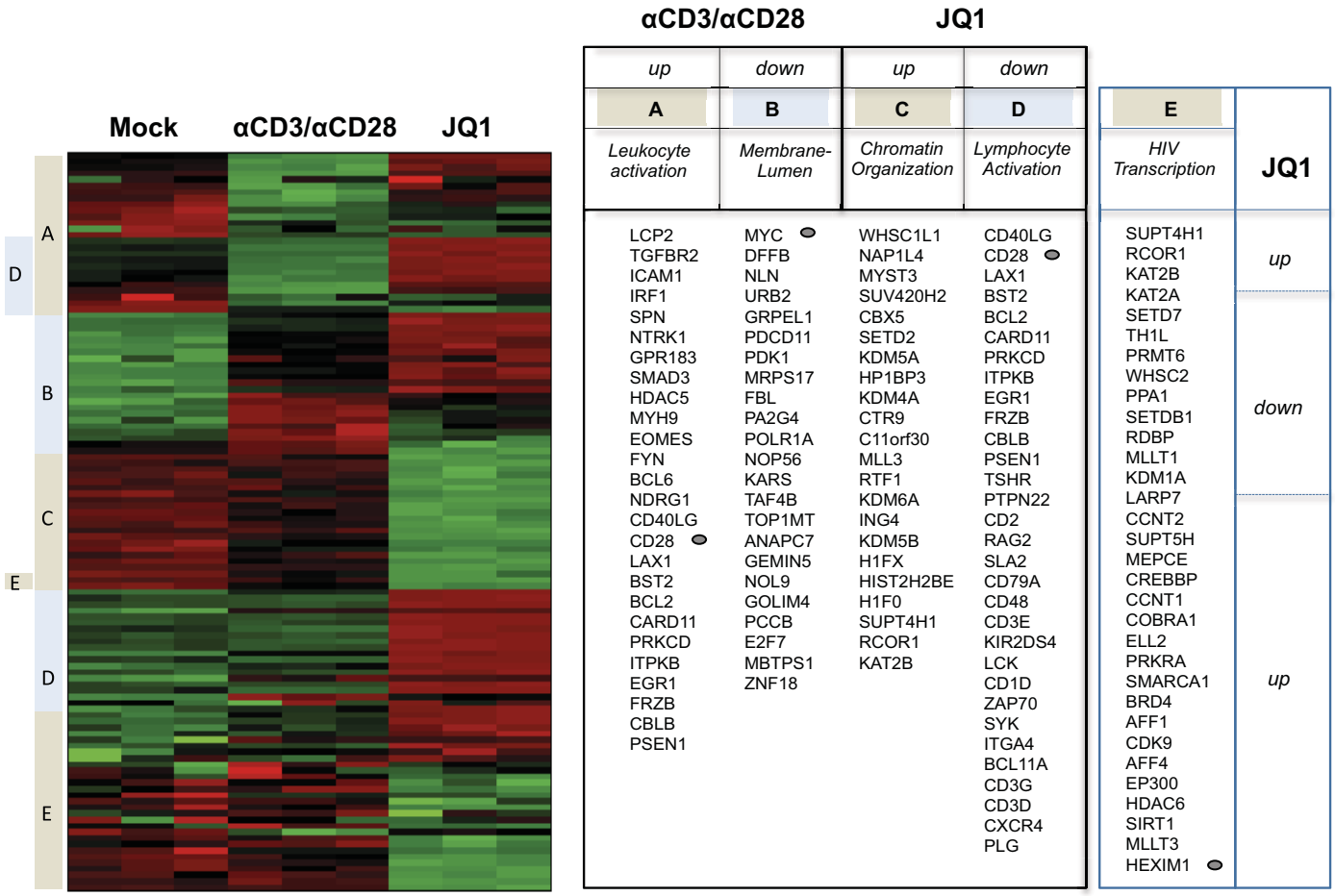

Figure 5. The top gene categories for induction and repression by α CD3/α CD28 and JQ1 in J-Lat T cells. Clusters A and B, respectively, indicate the top-induced GO category "leukocyte activation" and top-repressed GO category "membrane-enclosed lumen" with αCD3/αCD28 stimulation. Clusters C and D, respectively, indicate the top-induced GO category "chromatin organization" and top-repressed GO category "lymphocyte activation" with JQ1S stimulation. Cluster E indicates selected HIV-associated genes that were influenced by JQ1S treatment. Transcripts validated by qRT-PCR in J-Lat 10.6 cells are indicated with a dark oval (CD28, MYC, HEXIM1).

JQ1 but not αCD3/αCD28. The corepressor RE1 silencing transcription factor 1, which has been associated previously with HIV reactivation [49], was induced by JQ1 but not αCD3/αCD28. The class III deacetylase SIRT1, a gene associated with longevity and required for full Tat recycling and transactivation [50], was potently induced by JQ1 and not αCD3/αCD28. The Tat associated super-elongation complex subunits AFF4, AFF1, and AF9 were up-regulated by JQ1 but not αCD3/αCD28 (Fig. 5, Cluster E). In data not shown but indicated with dark ovals in Fig. 5, selected genes were chosen as targets for qRT-PCR validation of the microarray results to represent αCD3/αCD28-mediated up-regulation (CD28) or down-regulation (MYC) and JQ1-mediated up-regulation (HEXIM1) or down-regulation (CD28). In all cases, qRT-PCR results were significantly correlated with microarray results.

In conclusion, we describe the effects of a novel and selective BET family bromodomain inhibitor JQ1 on HIV-1 transcriptional reactivation. We show that JQ1 suppresses T cell proliferation, induces histone modification genes, and potently suppresses genes associated with T cell activation. Previously, the strategy of purging viral reservoirs focused on T cell activation using IL-2 and mitogenic αCD3 antibodies with limited success and prompting the need for alternative strategies [17, 18, 51]. There is substantial interest in the role of chromatin modification and control of the HIV LTR [52]. Studies looking at reactivation of latent virus using HDAC inhibitors to induce expression of the HIV-1 genome suggest that small molecules that can gain access to DNA and facilitate viral gene expression without inducing T cell activation might be promising [21, 24]. Thus, JQ1 appears to be a candidate with desirable features that could be used to supplement efforts to achieve viral eradication.

Based on the microarray data, JQ1 appears to promote chromatin reorganization by inducing the expression of histone acetytransferases, HDACs, and histone demethylases, while suppressing histone methyltransferases and multiple T cell activation genes. Many of the genes regulated by JQ1 are predicted to influence post-translational modifications of Tat [43], suggesting that JQ1 potentially reactivates HIV by enhancing Tat activity. JQ1 possibly promotes Tat activity by increasing Tat

recycling, repressing negative regulators such as methyltransferases, and increasing Tat activators such as acetyltransferases, supporting the view that Tat has co-opted signaling associated with histone organization as a distinct pathway from T cell stimulation to promote viral activation [16]. Whether this process is reliant upon JQ1-mediated inhibition of BRD4 will require further study.

The influence of JQ1 on global T cell transcription shares some features with previously described HDAC inhibitors TSA [40] and SAHA [42, 53]. Notably, TSA results in the altered expression of a subset of genes involved in T cell responses and the down-regulation of various costimulatory molecules such as CD28, important for T cell function [40]. SAHA coordinately regulates the expression of several genes within distinct apoptosis and cell-cycle pathways. Multiple genes within the Myc, cyclin, and apoptosis pathways are regulated by SAHA in a manner that favors apoptosis and decreased cellular proliferation, consistent with our observations for JQ1. SAHA, like JQ1, also exhibits anti-inflammatory properties [42, 53].

An intriguing link between histone modifications and T cell activation modulation by JQ1 is the observed up-regulation of SIRT1 in the J-Lat 10.6 cells. SIRT1 has been shown to deacetylate Tat protein in late activation and promotes Tat recycling to the HIV promoter, but SIRT1 also appears to be inhibited by Tat [50]. In addition, SIRT1 deacetylates the p65 component of NF-κB, RelA [54], negatively impacting NF-κB-driven gene expression, which may help to explain the observed down-regulation of genes downstream of TCR-mediated (e.g., αCD3/αCD28) activation. In addition, the direct target of JQ1, BRD4, recognizes acetylated RelA and promotes NF-κB-driven gene expression [55].

JQ1 also potently up-regulated HEXIM1 and suppressed Myc based on the array data, consistent with recent reports for JQ1 activity in B cells [56] and other cell types [41]. The up-regulation of HEXIM1 is of interest, as this protein sequesters the cyclin components of P-TEFb [57]. The down-regulation of Myc by JQ1 is intriguing in the context of HIV reactivation, as Myc is reported to recruit HDAC1 to promote latency [58]. Myc furthermore interacts with P-TEFb, although the implications of this activity on HIV latency are unclear, and further study will be required.

Collectively, in this study, we provide evidence that JQ1 has the potential to induce expression of latent HIV. The mechanism of action through which JQ1 activates HIV remains unclear but is likely to differ from T cell activation pathways and may involve modulation of Tat regulators and/or BRD4 activity at the HIV promoter and will require further study. This compound, or derivatives thereof, may assist in therapeutic strategies focused on viral eradication and in efforts to better understand regulatory pathways controlling HIV expression and reactivation.

AUTHORSHIP

C.B., A.C.B., G.V.D., D.M.M., N.A., and M.M. wrote the paper. C.B., A.C.B., D.M., N.A., and P.S. performed the research. J.B. provided the JQ1 reagent. M.M. designed research and established the concept of the study.

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