

INSIGHTS

Inhibiting BRD4 to generate BETter T cell memory

Moujtaba Y. Kasmani¹ and Weiguo Cui²

BRD4 is a bromodomain-containing protein that binds acetylated histones to regulate transcription. In this issue of JEM, Milner et al. (2021. *J. Exp. Med.* <https://doi.org/10.1084/jem.20202512>) show that BRD4 plays a critical role in the effector function of CD8 T cells responding to infection and cancer.

CD8 T cells respond to acute infection by differentiating into short-lived terminal effector (TE) cells or long-lived memory precursor (MP) cells (Kaeche and Cui, 2012). Factors that affect the development of these two differentiated T cell subsets are highly sought after, as improved T cell memory formation has a significant impact on vaccine efficacy (Ahlers and Belyakov, 2010). Various transcription factors (TFs) such as T-bet (Joshi et al., 2007) and Eomes (Banerjee et al., 2010) are important for the function and cell fates of TE and MP cells, respectively. Work by Milner et al. (2021) in this issue of JEM sheds further light on the pathways regulating TE and MP cell formation by revealing a critical role for the bromodomain-containing protein BRD4 in the formation of TE CD8 T cells.

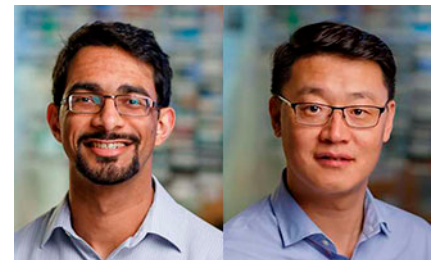
BRD4 is a member of the bromodomain and extraterminal domain (BET) family of proteins (BRD2, BRD3, BRD4, and BRDT), which bind acetylated lysine residues of histones via two eponymous bromodomains in tandem (Dey et al., 2019). By binding histones, particularly those located in super-enhancers, BRD4 serves as a scaffold to assemble transcriptional complex machinery in a variety of cell types (Dey et al., 2019).

Using an in vivo RNAi screen, Milner et al. showed that BRD4 is critical for the generation of TE cells during the effector phase of acute lymphocytic choriomeningitis virus (LCMV) infection. Further RNAi experiments using adoptively transferred CD8 T cells revealed that this decrease in

effector-like cells persists during the memory phase following viral clearance, as *Brd4* knockdown also led to a decreased frequency of terminally differentiated effector memory CD8 T (t- T_{EM}) cells, which exhibit greater cytotoxicity but lower recall capacity than conventional T_{EM} cells (Milner et al., 2020). Conversely, *Brd4* knockdown led to an increased proportion of central memory CD8 T (T_{CM}) cells. The authors then supplemented these experiments by using an inducible deletion mixed bone marrow chimera model to verify that loss of BRD4 intrinsically affects CD8 T cell differentiation.

Given the dramatic impact of *Brd4* RNAi, the authors then investigated if BRD4 can be targeted at the protein level using small molecule inhibitors. The pan-BET bromodomain inhibitor JQ1 prevents all four BET proteins from binding acetylated lysine residues by blocking these proteins' bromodomains (Boi et al., 2015; Filippakopoulos et al., 2010). Indeed, daily in vivo administration of JQ1 or other bromodomain inhibitors caused a decrease in TE CD8 T cells and an increase in MP CD8 T cells by day 5 after LCMV infection, similar to RNAi results.

To gain mechanistic insights, the authors performed bulk RNA sequencing (RNA-seq) on early effector cells (EECs), the precursors to both TE and MP cells, on day 5 after LCMV infection. Intriguingly, the vast majority of genes up-regulated or down-regulated by *Brd4* RNAi were also up-regulated or down-regulated, respectively, by the pan-BET bromodomain inhibitor JQ1, suggesting that



Insights from Moujtaba Y. Kasmani and Weiguo Cui.

BRD4 plays a larger role in controlling CD8 T cell differentiation during viral infection than do the other BET proteins.

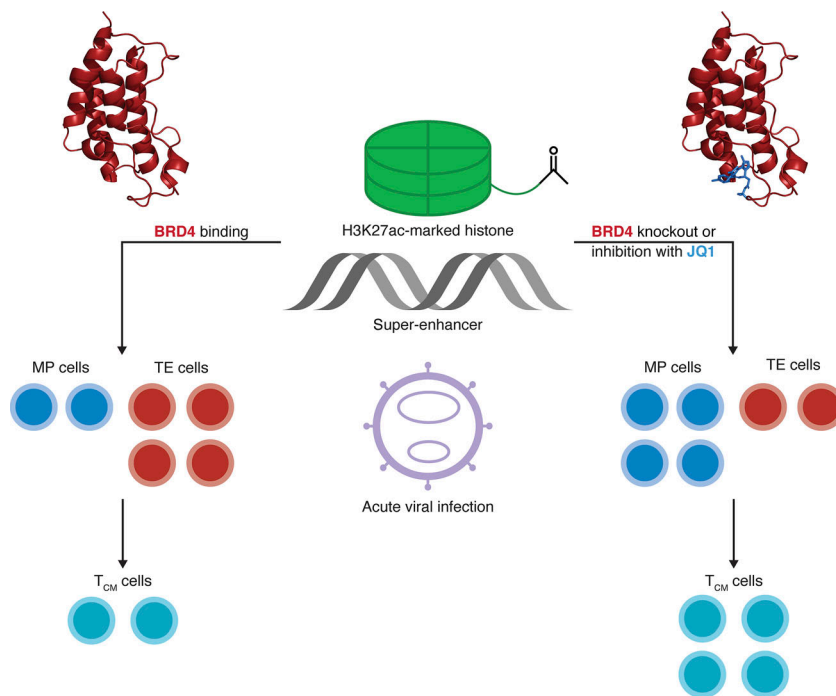
To expand upon these transcriptional differences, a separate bone marrow chimera model was used that combined WT marrow and marrow from estrogen receptor-Cre *Brd4*^{fl/fl} mice, which allowed for inducible deletion of *Brd4* on days 5–8 after infection. Bulk RNA-seq revealed that ablation of *Brd4* drastically reduced the expression of signature genes in EEC, TE, and MP cells compared with WT controls, suggesting that BRD4 inhibition impacts the lineage stability of these CD8 T cell phenotypes. However, differences seemed most pronounced in TE cells, as *Brd4* knockout TE cells expressed a significantly higher gene set enrichment analysis score for MP and EEC gene signatures compared with WT TE cells.

As the BRD4 bromodomains bind acetylated lysine residues (Dey et al., 2019), chromatin immunoprecipitation sequencing

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The pan-BET protein inhibitor JQ1 inhibits the binding of BRD4 to acetylated lysine residues of histones near super-enhancers. BRD4 inhibition or knockout during acute viral infection skews CD8 T cell differentiation toward an MP fate rather than a TE fate, leading to an increased proportion of T_{CM} cells following viral clearance. Protein structure adapted from Protein Data Bank (ID: 3MXF; [Filippakopoulos et al., 2010](#)).

(ChIP-seq) was performed to investigate overlap between BRD4 binding sites and the presence of H3K27ac histone modifications. Overlap between BRD4 binding and H3K27ac modification was seen in genes important for TE cell function, including the transcription factor *Id2* and the chemokine receptor *Cx3cr1*. Milner et al. further characterized the epigenetic functions of BRD4 by analyzing ChIP-seq data collected from TE cells to investigate the binding of BRD4 to super-enhancers, genomic regions containing several enhancers in close proximity ([Milner et al., 2021](#); [Lovén et al., 2013](#)). Intriguingly, ChIP-seq data demonstrated that BRD4 binds to 549 of 554 super-enhancers in TE cells, mirroring the known binding of BRD4 to super-enhancers in other cell types ([Dey et al., 2019](#); [Lovén et al., 2013](#)). BRD4-bound TE super-enhancers were located close to genes important for TE function, including *Gzmb* and *Klrg1*. Collectively, these data suggest that the ability of BRD4 to bind to TE-specific super-enhancers is critical for TE cell function (see figure).

As BRD4 knockdown inhibited terminal differentiation of CD8 T cells in viral infection, the authors reasoned that BRD4

knockdown may prevent terminal exhaustion of CD8 tumor infiltrating CD8 T lymphocytes (TILs; [Ahmadzadeh et al., 2009](#)). Indeed, both shRNA-mediated BRD4 knockdown and JQ1 administration caused a decrease in the proportion of terminally exhausted CD8 T cells in a B16 murine melanoma model. Curiously, the authors found that dual treatment with adoptively transferred tumor-specific CD8 T cells and JQ1 resulted in worse tumor control than adoptive cell transfer (ACT) alone. This suggests that BET inhibitor dosage or tumor type may negatively impact the therapeutic function of BET protein inhibition in the context of ACT, a finding which has implications for clinical cancer trials that may aim to use BET inhibition ([Khandekar and Tiriveedhi, 2020](#)) in conjunction with ACT such as chimeric antigen receptor T cell therapy. Conversely, co-administration of JQ1 and anti-PD-1 resulted in improved tumor control compared with either therapy alone in an MC38 colon cancer model. ChIP-seq performed on TE cells from acute LCMV infection revealed that BRD4 binds H3K27ac-marked enhancers near exhaustion-related genes such as *Havcr2*, which encodes the inhibitory receptor Tim3. This suggests that

the epigenetic functions of BRD4 in TILs mirror its role in TE cells in acute viral infection, as it promotes terminal differentiation of CD8 T cells in both settings.

The work published by [Milner et al. \(2021\)](#) in this issue of *JEM* sheds light on the role of BRD4 in the function and differentiation of CD8 T cells. Yet, as is always the case in science, every answer also raises more questions. A natural question to ask is how the authors' findings apply to other well-studied models of infection and inflammation. In particular, several recent papers have demonstrated the existence of a subset of cytolytic effector cells in chronic infection ([Zander et al., 2019](#); [Hudson et al., 2019](#); [Chen et al., 2019](#); [Beltra et al., 2020](#)). It would be intriguing to test whether BRD4 inhibition would impact effector CD8 T cell differentiation and viral control in this context, as chronic infection serves as a sort of middle ground between the effector response seen in acute viral infection and T cell exhaustion seen in tumors. Moreover, the ability of BRD4 inhibition to curtail effector CD8 T cell responses may prove to be a valuable therapeutic strategy in the context of autoimmune conditions such as type 1 diabetes ([Fu et al., 2014](#)), wherein suppression of effector T cells improves clinical outcomes ([Herold et al., 2019](#)).

The ability of BRD4 inhibition to bolster memory CD8 T cell formation in acute viral infection could also be explored in the context of tissue-resident memory (T_{RM}) CD8 T cells, which are especially important for protection against pathogens such as influenza virus ([Pizzolla et al., 2017](#)). This could broaden the potential applications of BRD4 inhibition into influenza vaccine development, as a major goal of influenza vaccines is to generate protective T_{RM} CD8 T cells ([Pizzolla and Wakim, 2019](#)).

Finally, one avenue in need of further study is the link between cellular metabolism and the ability of BRD4 to bind acetylated lysine residues. Lipids can serve as a carbon source for histone acetylation ([McDonnell et al., 2016](#)), and BRD4 can bind to histones acetylated by acyl-CoA metabolites generated by fatty acid metabolism ([Olp et al., 2017](#)). Fatty acid oxidation is preferentially used by TILs, in part due to glucose consumption by tumor cells ([Lim et al., 2020](#)) and in part due to T cell-intrinsic metabolic changes caused by PD-1 and STAT3 signaling ([Zhang et al., 2020](#)).

It would therefore be valuable to explore the link between fatty acid oxidation, histone acetylation, and BRD4 binding in the setting of cancer. In short, work by [Milner et al. \(2021\)](#) in this issue of *JEM* has unveiled possibilities for new modes of therapeutic immunomodulation in acute and chronic infections, cancer, and autoimmunity.

Disclosures: The authors declare no competing financial interests exist.

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