

## INVITED REVIEW

# Exploring epigenetic reprogramming during central nervous system infection

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**Abstract**

Epigenetics involves the study of various modes of adaptable transcriptional regulation, contributing to cell identity, characteristics, and function. During central nervous system (CNS) infection, epigenetic mechanisms can exert pronounced control over the maturation and antimicrobial properties of nearly every immune cell type. Epigenetics is a relatively new field, with the first mention of these marks proposed only a half-century ago and a substantial body of immunological epigenetic research emerging only in the last few decades. Here, we review the best-characterized epigenetic marks and their functions as well as illustrate how various immune cell populations responding to CNS infection utilize these marks to organize their activation state and inflammatory processes. We also discuss the metabolic and clinical implications of epigenetic marks and the rapidly expanding set of tools available to researchers that are enabling elucidation of increasingly detailed genetic regulatory pathways. These considerations paint an intricate picture of inflammatory regulation, where epigenetic marks influence genetic, signaling, and environmental elements to orchestrate a tailored immunological response to the threat at hand, cementing epigenetics as an important player in immunity.

**KEYWORDS**

acetylation, central nervous system, epigenetics, histone, infection, methylation

## 1 | INTRODUCTION

Nearly 65 years ago, Francis Crick first proposed what would become the central dogma of biology.<sup>1</sup> That is, all life processes are derived from the flow of information between macromolecules—from DNA, to RNA, and protein. While this concept has become more nuanced over time because of unique regulatory processes at each step, it remains a staple of the central framework of biological thought. Around this same time, hematopoietic stem cells, derived from the Greek “to produce blood,” were identified for their ability to generate the diverse lineages of blood and immune cells.<sup>2</sup> Both

discoveries answered large questions in biology but stimulated many more. How do immune cells with the same DNA achieve dramatically different lineages and phenotypes? How do cells decide which genes to utilize at a particular time? And more generally, how are the approximately three-billion base pairs of the human genome<sup>3</sup> organized so they can be accessed for use when needed? The answer to these questions, in part, is via epigenetics.

Epigenetics involves chemical modification of the genome in ways which change its function without altering the core DNA sequence. This is achieved with the addition or removal of small molecular moieties on either the DNA itself or histones, proteins that

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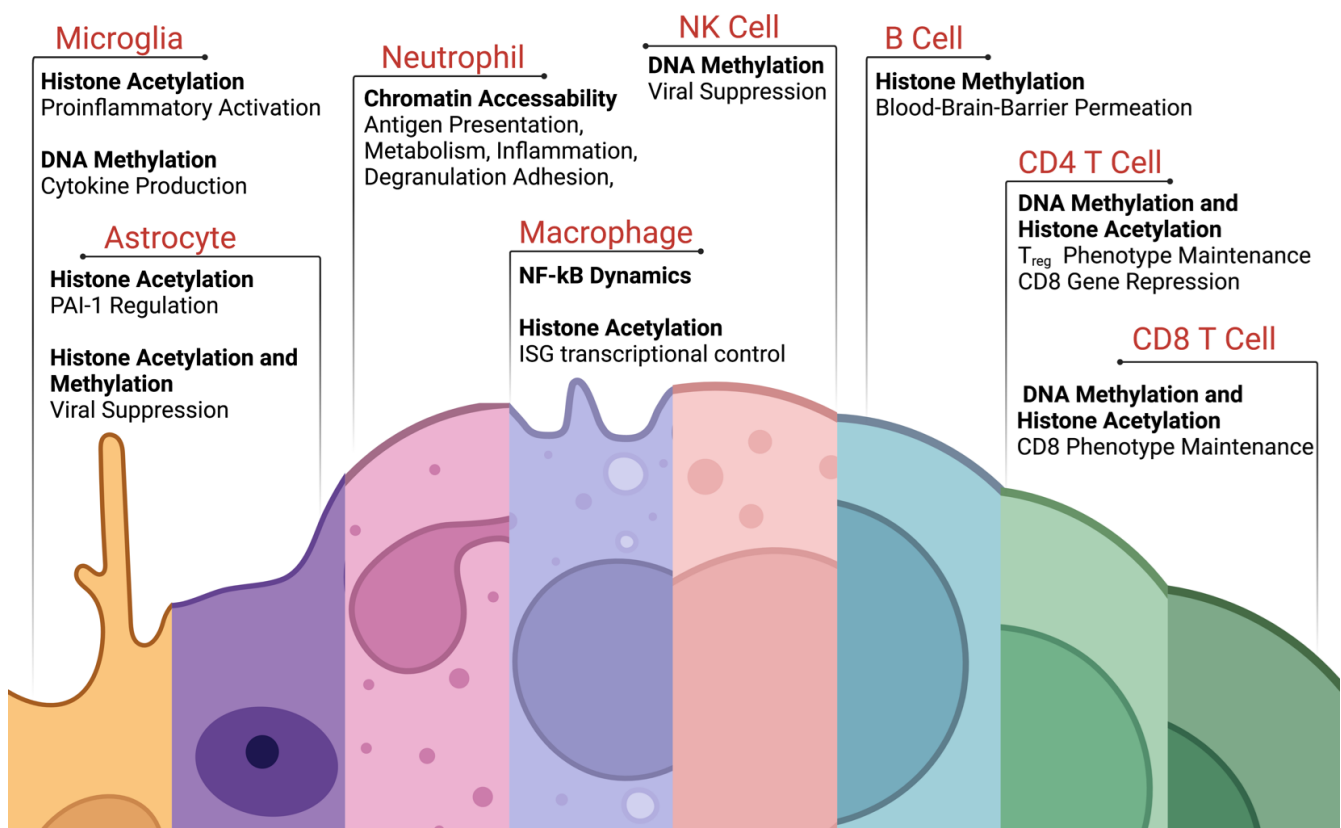
wrap DNA for genomic organization.<sup>4</sup> These moieties, or epigenetic marks, can be derived from a wide range of metabolic byproducts, with acetylation (derived from acetyl-CoA) and methylation (derived from methyl-group transferring compounds) being the most well studied.<sup>4,5</sup> Alternative epigenetic mechanisms, such as micro-RNA-mediated silencing of mRNA transcripts, are a growing field of research,<sup>6</sup> but are beyond the scope of this review. Instead, we focus on direct DNA and histone modifications and how these processes are intimately tied to proper functioning (and sometimes dysfunction) of the immune system during central nervous system (CNS) infection. The study of epigenetics is expansive and rapidly evolving; however, there is much left to be uncovered regarding the role of epigenetic remodeling during infection, especially within the CNS. Therefore, this review will discuss work from both peripheral and CNS infection models, applying concepts uncovered in other organ systems and in vitro to the brain, and speculating on which phenomena may hold true in the unique microenvironment of the CNS. Accordingly, this review is not meant to represent an exhaustive report of recent epigenetic findings, but rather an illustrative guide of the many ways that epigenetic mechanisms orchestrate immune responses within the CNS at various stages of infection.

Throughout this review, we will explore the intimate relationship between changes in the epigenetic landscape and how this affects immune cell phenotype and function in response to infection. The rapid kinetics of epigenetic marks are of special significance to the

immune system, as cells are required to quickly shift from resting to activated states upon encountering noxious stimuli. Additionally, the reversibility of many epigenetic mechanisms provides the immune system with an attractive mode of regulation that can be effectively titrated or negated upon resolution of inflammatory stimuli, limiting collateral tissue damage. Accordingly, the immune system has evolved to extensively utilize epigenetic modifications, with examples described in nearly every immune cell type.<sup>7,8</sup> We will first review the biochemistry and mechanics of common epigenetic marks and address how each is regulated. Next, we will explore examples of these marks through the lens of cell types that respond to CNS infection, beginning with glia and resident innate immune cells of the brain and progressing to infiltrating innate and adaptive immune populations (Figure 1). Finally, we will address new technologies that can be applied to better understand epigenetic changes that influence CNS infection as well as speculate on the direction of the field of epigenetics, clinical applications, and emerging areas of research.

## 1.1 | Acetylation

Histone acetylation (HAc) is the process of covalently modifying histone lysine residues with acetyl groups.<sup>5</sup> Histones are positively charged octameric complexes, consisting of two copies of four different histone proteins, H2A, H2B, H3, and H4. Negatively charged



**FIGURE 1** Cell type-specific effects of epigenetic modification. Separated by cell type and mark, all major immune cell populations responding to CNS infection exhibit epigenetic control over their differentiation and/or effector functions. Figure created with BioRender.com

DNA is electrostatically attracted to the positive histone octamer, wrapping around a histone core and forming a structure called a nucleosome.<sup>5</sup> On a genomic scale, this winding of DNA around histones produces tightly packed tertiary structures for greater genomic organization and regulation.

As the name implies, acetylation marks are derived from acetyl-CoA and by extension, epigenetic processes are sensitive to fluctuations in nutrient availability or metabolic reprogramming.<sup>4,9</sup> Cells produce acetyl-CoA via three major metabolic pathways, namely glycolysis,  $\beta$ -oxidation of fatty acids, and acetate metabolism. Glycolysis is considered the primary source for acetylation materials under homeostatic conditions, with the other mechanisms becoming important during times of nutrient and/or cellular stress. The details of these metabolic pathways in relation to chromatin modifications have been thoroughly reviewed elsewhere.<sup>10</sup> Interestingly, there is growing evidence for distinct cytoplasmic and nuclear acetyl-CoA pools, suggesting that acetyl-CoA availability may have a spatial component, affecting protein vs. histone acetylation, respectively.<sup>10</sup> Further, histone acetylation may be aided by liquid-liquid phase separation, referring to localized pockets of high acetyl-CoA levels near genomic sites undergoing active epigenetic reorganization in the face of low total nucleoplasmic acetyl-CoA concentrations, allowing a mass effect to favor acetylation mark placement.<sup>4</sup> This is an emerging concept, and the reader is directed to a recent review by Dai et al<sup>4</sup> on the topic. Collectively, there is a well-documented relationship between acetyl-CoA production, metabolism, and global histone acetylation capacity in numerous cell types.<sup>9</sup>

Protruding from the histone core are polypeptide tails containing a high abundance of positively charged lysine residues that enhance electrostatic interactions between histones and DNA. Importantly, lysine residues can be modified by acetylation, which is catalyzed by a class of epigenetic "writer" enzymes called histone acetyltransferases (HATs). Using acetyl-CoA as a substrate, these enzymes attach an acetyl group to histone lysine residues, releasing coenzyme A and a newly acetylated histone<sup>4</sup> (Figure 2). Once placed, these modifications are thought to have two synergistic effects on gene transcription. First, acetylation neutralizes the positive charge of a lysine residue, loosening the attractive force between histone and DNA. This favors a genomic conformation termed "open chromatin" or euchromatin, as this relaxed state reduces steric hindrance and allows transcription factors and RNA polymerase greater spatial access to the DNA strand. The net effect is enhanced gene transcription or access to an enhancer surrounding the acetylation marks.<sup>5</sup> Second, acetylation acts as a chemical flag that directs the action of other nuclear enzymes. The archetypal example of this process involves epigenetic "reader" domains (which detect acetylation marks) called bromodomains (BRD). BRDs are evolutionally conserved protein domains that bind acetylated histones as a part of a multi-protein complex to direct associated proteins to acetylated regions of the genome for localized action.<sup>11</sup> Examples of BRD-containing proteins include the bromodomain and extraterminal domain (BET) family members BRD2, BRD3, BRD4, and BRDT.<sup>12</sup> BETs can associate with a diverse group of proteins, including transcription factors,

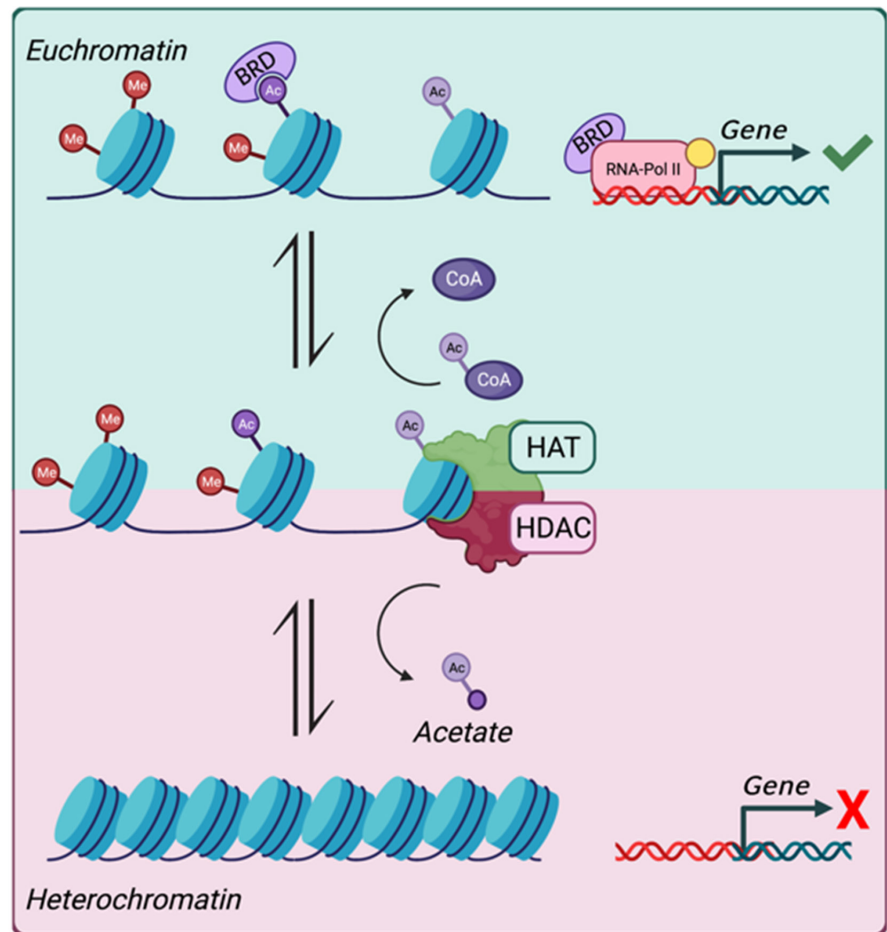
helicases, methyltransferases, HATs, histone deacetylases, and RNA polymerase.<sup>13</sup> The architecture and chemical function of BRD proteins have been reviewed elsewhere.<sup>11</sup> Collectively, increased gene transcription mediated by histone acetylation is influenced by the coordinated action of acetylation-induced euchromatin and the direction of other nuclear proteins to these sites. However, the behavior of acetylation marks can differ widely depending on the specific lysine modified. For example, H3 histone acetylation of lysine 27 (H3K27ac) is associated with euchromatin.<sup>14</sup> However, acetylation at a different residue on the same histone tail may produce different effects.

The rapidly reversible nature of the acetylation system relies on a delicate balance between HATs, which place acetylation marks, and another group of enzymes responsible for the removal of histone acetyl groups called histone deacetylases (HDACs).<sup>5</sup> This class consists of 18 different HDAC proteins divided into four distinct classes based on function and sequence homology. Class I, II, and IV HDACs are zinc-dependent and can deacetylate cytosolic proteins or histones, the specificities of which differ for each HDAC enzyme.<sup>13</sup> Like the other HDACs, class III HDACs (sirtuins) may deacetylate cytosolic proteins or histones. However, these enzymes are distinct from the other classes as they are NAD<sup>+</sup>-dependent and play a significant role in regulating mitochondrial function.<sup>15</sup> Deacetylation occurs when an HDAC enzyme cleaves the acetyl group from its associated lysine residue, releasing acetate as a byproduct. This restores the positive charge to lysine and favors tighter histone-DNA binding, promoting "closed chromatin" or heterochromatin that restricts transcriptional access to the underlying DNA.<sup>4</sup> By extension, HDACs are considered epigenetic "erasers" and complete the lifecycle of an acetylation mark. Importantly, HDACs do not work indiscriminately or without cellular guidance. A single HDAC enzyme may be a catalytic component of many different multi-protein genetic regulatory complexes with distinct functions and binding sites. This can complicate efforts to understand the function of a particular HDAC. While physical deacetylation of a histone is associated with gene repression, recruitment of HDACs to their intended loci is a multifactorial and poorly understood process. As another layer of complexity, inhibition or reduced expression of a single HDAC enzyme may have multifaceted effects on epigenetic regulation if it participates in distinct complexes with potentially antagonistic functions.<sup>13</sup>

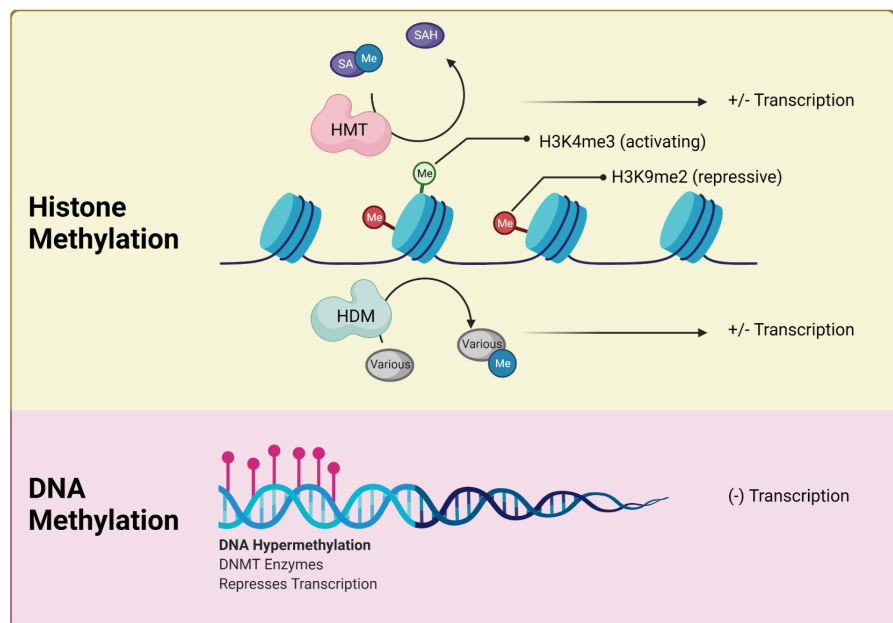
## 1.2 | Methylation

Methylation is another well-studied epigenetic modification that can occur either directly on the DNA strand or histones. Importantly, modifications of these two targets are not synonymous and can have different effects on the cellular transcriptome. Like acetylation, methylation marks are reliant on nutrient availability and metabolic activity within the cell.<sup>4</sup> Briefly, methylation relies on exogenous methionine uptake, which is then converted into S-adenosyl methionine (SAM). This metabolite is demethylated to form S-adenosyl homocysteine (SAH) by methyltransferase enzymes, where the

**FIGURE 2** Overview of the epigenetic acetylation system. Histone acetyltransferases (HATs) utilize acetyl-CoA for formation of acetylation marks, leading to euchromatin formation, reader protein binding, and favoring gene transcription. Histone deacetylases (HDACs) remove acetylation marks, favoring heterochromatin and repressing transcription. Figure created with BioRender.com



**FIGURE 3** Overview of epigenetic methylation systems. Histone methylation is controlled by the balance of histone methyltransferase (HMT) and histone demethylase (HDM) activity, which add and remove methyl groups on histones, respectively. This may lead to transcriptional activation or repression, depending on the specific modified histone residue. DNA methylation is mediated by DNA methyltransferase (DNMT) enzymes and represses transcription. Figure created with BioRender.com



liberated methyl groups are added to proteins/DNA.<sup>4</sup> Originally, histone methylation was thought to be a permanent modification, owing to early measurements of identical half-lives between histones and methylation marks<sup>16</sup>; however, this is now known to be a dynamic process. Like the acetylation system, histone methylation

is controlled by the balanced action of methylation writer and eraser enzymes. Methylation writers, or histone methyltransferases (HMTs), use SAM to add methyl groups on histone lysine or arginine residues, predominantly on H3 and H4 proteins<sup>4</sup> (Figure 3). While acetylation marks are generally associated with increased gene

transcription, mono-, di-, or tri- methylation can occur on a single amino acid residue and either repress or activate transcription depending on the location.<sup>6</sup> For example, trimethylation of lysine four on the H3 subunit (H3K4me3) is associated with increased transcriptional activity,<sup>17</sup> while dimethylation of lysine nine on H3 (H3K9me2) leads to robust transcriptional repression.<sup>18</sup> The removal of methylation marks is performed by histone demethylase (HDM) enzymes. Two different classes of HDMs have been identified that exhibit preferences for distinct methylation marks and catalyze slightly different reactions. The final demethylation step yields a molecule of formaldehyde as a byproduct.<sup>4</sup>

### 1.3 | DNA Methylation

DNA methylation operates distinctly from histone methylation in both the placement and use of methylation marks (Figure 3). Both processes use the same cofactor (SAM), but DNA methylation occurs directly on the carbon ring of cytosine.<sup>4</sup> Most often, this occurs at genomic loci referred to as CpG islands, where a cytosine base is directly followed by guanine in the DNA sequence. Also distinct from histone methylation, DNA methylation is thought to universally repress transcription.<sup>4</sup> Three main enzymes catalyze the placement of DNA methylation marks, namely DNA methyltransferase 1 (DNMT1) that acts as a maintenance methyltransferase, and DNMT3a and DNMT3b, which are thought to function as reactive methyltransferases that respond to cellular conditions and can alter transcriptional programs.<sup>19</sup> DNA methylation marks inhibit transcription by sterically obstructing DNA and recruiting proteins associated with transcriptional repression.<sup>20</sup> 5-methylcytosine bases function as a binding site for DNA methylation reader proteins, such

as methyl CpG binding protein 2 (MeCP2), which directs the action of HDACs and other nuclear enzymes.<sup>19</sup> DNA demethylation can occur through two different pathways. The first is active enzymatic demethylation which occurs through a complex series of reactions and has been reviewed elsewhere.<sup>21</sup> In the second, methylation marks may be lost passively during cellular replication by the failure to remethylate the newly synthesized DNA strand.<sup>21</sup>

### 1.4 | Acylation and exotic marks

In addition to the well-characterized epigenetic processes of acetylation and methylation, numerous studies have identified a spectrum of other metabolites capable of modifying DNA and histones, broadly referred to as acylation. For a detailed overview of these exotic epigenetic marks, we direct the reader to a recent excellent review on the topic.<sup>4</sup> Some examples of these marks are included in Table 1. As is the case with acetylation and methylation, many of these reactions are not histone-specific and occur on a wide range of intracellular proteins. Additionally, acylation marks may compete with acetylation and methylation for the same amino acid residues as well as interact with other marks.<sup>4</sup> Most acylation marks have been observed when substrate concentrations in the nucleoplasm are elevated, which can induce changes in cellular behavior<sup>22-24</sup>; however, the extent of these effects remains to be determined. One such example is the addition of a lactyl group to histone lysine residues, termed lactylation. In macrophages, bacterial exposure promotes glycolytic flux, generating high amounts of lactate as a byproduct. Zhang et al demonstrated that lactate accumulation generated lactylation marks in a pattern distinct from acetylation.<sup>25</sup> Further, histone lactylation induced the expression of genes involved in wound

TABLE 1 Examples of exotic epigenetic marks and their described functions

Exotic mark	Description	Function	Source
Succinylation	Derived from succinyl-CoA	Transcriptional Activation	22
Malonylation	Derived from citrate metabolism	Can regulate chromatin segregation in the nucleus	23,125
Benzoylation	Derived from sodium benzoate, a chemical food preservative	Transcriptional Activation, SIRT2 involved in removal	24
Crotonylation	Derived from crotonyl-CoA produced by SCFA <sup>a</sup> crotonate	Transcriptional Activation	126
Lactylation	Derived from lactate metabolism	Transcriptional Activation	25,127,128
Homocysteinylation	Derived from homocysteine	Can interact with methylation and acetylation marks	129
Monoaminylation	Derived from serotonin and dopamine	Can interact with methylation marks	130
ADP-ribosylation	Derived from ADP-ribose <sup>b</sup> polymers	Involved in DNA damage and cell cycle regulation	131
O-GlcNacylation	Derived from O-linked $\beta$ -N-acetylglucosamine	Shown to fluctuate during mitosis and heat shock conditions	132
$\beta$ -hydroxybutyrylation	Derived from $\beta$ -hydroxybutyrate	Produced during fasting, can activate starvation responses	133

<sup>a</sup>Short-chain fatty acid.

<sup>b</sup>Adenosine diphosphate-ribose.

healing (ie, arginase-1) in macrophages after prolonged proinflammatory activity, suggesting this mark may play an important role in the transition to an anti-inflammatory state.<sup>25</sup> In the adaptive immune system, lactate dehydrogenase A (LDHA) activity modulates H3 acetylation in CD4<sup>+</sup> T cells via lactate to promote Th<sub>1</sub> responses and IFN- $\gamma$  production.<sup>26</sup> Our laboratory has recently identified yet another epigenetic role for lactate during biofilm infection, where *Staphylococcus aureus* (*S. aureus*)-derived lactate can affect the host epigenome by inhibiting HDAC11, a known negative regulator of HDAC6.<sup>27,28</sup> As a consequence, unchecked HDAC6 activity leads to enhanced IL-10 production in granulocytic myeloid-derived suppressor cells (G-MDSCs) and macrophages that promotes biofilm persistence in a mouse model of prosthetic joint infection.<sup>29</sup> The study of exotic histone marks during infection is in its infancy and represents an exciting area for future epigenetic and immunological investigation.

## 1.5 | Metabolism, immunology, and epigenetic complexity

In recent years, there has been a resurgence in exploring the link between metabolism and immune cell function, referred to as immunometabolism.<sup>30,31</sup> In terms of infectious disease, our laboratory has shown that reprogramming monocyte/macrophage metabolism from an oxidative to glycolytic phenotype in vivo can promote *S. aureus* biofilm clearance.<sup>32</sup> Likewise, bacterial-derived metabolites can alter the epigenome of infiltrating leukocytes to favor anti-inflammatory responses to ensure biofilm persistence.<sup>29</sup> In the next section, we will review how epigenetic changes can influence the immune response to CNS infection and identify where pathogens may target these pathways (Figure 1). We will first discuss microglia and astrocytes as the initial responders to CNS infection and explore examples of how epigenetic changes regulate glial activation, including their ability to recruit peripheral leukocytes to the infectious milieu. Next, we will explore epigenetic modifications in innate and adaptive immune cell populations that enter the infected CNS, concluding with a discussion on the role of epigenetics in CNS infection clearance and inflammatory resolution.

## 2 | FIRST RESPONDERS TO CNS INFECTION

### 2.1 | Microglial epigenetics

Microglia are the resident mononuclear phagocytes in the brain parenchyma and play a key role in sensing CNS infection. Microglia are derived from the yolk sac during embryogenesis<sup>33</sup> and remain distinct from peripheral monocyte lineages throughout life.<sup>34</sup> Although they share many attributes with macrophages, microglia possess unique capabilities and serve specialized functions within the CNS.<sup>34</sup> Microglia comprise around 10% of the cells in the brain

parenchyma<sup>35</sup> and proliferate slowly under steady-state conditions, or rapidly in response to inflammatory stimuli (infection, proinflammatory cytokines, tissue damage, etc)<sup>33</sup> to repopulate the compartment with no contribution from bone marrow-derived monocytes.<sup>34</sup>

The distinct origin of microglia as well as the unique microenvironment of the CNS necessitates specific investigation of microglial epigenetic activity separate from bone marrow-derived macrophages. While CNS-infiltrating macrophages and microglia exhibit some overlap in gene expression upon activation,<sup>36</sup> discrete surface marker expression and transcriptional differences allow for discrimination between the cell types.<sup>35</sup> Functionally, microglia can exert either pro- or anti-inflammatory roles in the brain corresponding to polarization along an inflammatory spectrum.<sup>37</sup> Investigation into epigenetic changes has proven to be a powerful tool to explain aspects of these microglial phenotypes. Below, we will discuss examples of how acetylation and methylation guide microglial activity during infection.

Acetylation appears to be especially important in regulating microglial proinflammatory responses. Various groups have demonstrated that BRD4, an epigenetic acetylation reader protein, is crucial for inducing positive transcription elongation factor b (pTEFb)- and nuclear factor- $\kappa$ B (NF- $\kappa$ B)-dependent genes such as nitric oxide synthase 2 (*NOS2*), *IL1B*, *IL6*, tumor necrosis factor (*TNF*), and monocyte chemoattractant protein-1 (*CCL2*).<sup>38,39</sup> DeMars et al<sup>40</sup> demonstrated robust induction of these proinflammatory genes in microglia in response to lipopolysaccharide (LPS), which was attenuated following targeted proteasomal degradation of BRD2 and BRD4. This study was one of the first to demonstrate a role for BET proteins as a link between microglial Toll-like receptor and NF- $\kappa$ B activation and increased RNA polymerase II activity. Importantly, this inflammatory role for BRD4 may be a broadly generalizable response across cell types, as we will later discuss BRD4 mechanics in regulating interferon (IFN)-stimulated genes during macrophage activation. The significance of acetylation reader proteins modulating microglial inflammatory gene expression is twofold. First, it illustrates the complexity of epigenetic transcriptional activation, as the placement of acetylation marks alone is not sufficient for a maximal inflammatory response. Second, it identifies additional layers of regulation over immune activation following pathogen exposure, the sum of which allow inflammation to be controlled with surgical precision.

Methylation also plays a key role in the inflammatory response of microglia, especially during senescence. With advancing age, the brain acquires a more progressive proinflammatory profile, yet the ability of microglia to respond to infectious insults becomes impaired. Evidence suggests dysregulation of SIRT1, a class III HDAC, as complicit in this phenomenon.<sup>41</sup> Cho et al reported a progressive decrease in SIRT1 expression in microglia of aging mice with concurrent increases in IL-1 $\beta$  production. SIRT1-deficient mice mimicked this trend and exhibited impaired spatial memory and increased markers of cellular senescence. Interestingly, SIRT1 is a known activator of DNA methyltransferases (DNMTs) that control DNA methylation. Both SIRT1 deficiency and DNMT inhibition resulted in hypomethylation upstream of the *IL1B* transcriptional start site and

significantly increased gene transcription.<sup>41</sup> Therefore, a reduction in SIRT1 during aging results in decreased methylation and increased basal IL-1 $\beta$  transcription, likely contributing to the proinflammatory milieu of the aging brain. As an extension of this work, Matt et al<sup>19</sup> showed decreased DNMT transcripts and increased IL-1 $\beta$  production in response to microglial challenge with LPS, a trend that was exaggerated in aged mice. Notably, one of the affected enzymes was DNMT3a, a methyltransferase responsible for shifting transcriptional programs. Collectively, these findings suggest that aging microglia are hyperinflammatory, both at baseline and in response to bacterial stimuli due, in part, to changing methylation landscapes. This is an epigenetic phenomenon that could play a key role in explaining increased CNS infection susceptibility and poor outcomes in the elderly.<sup>42</sup> An ongoing clinical analysis in our laboratory appears to agree with this possibility, where increased age was associated with a greater-than-average risk of surgical-site infection following a craniotomy neurosurgical intervention (unpublished data). This association is correlative at present, but skewed microglial epigenetic regulation may represent an important factor for promoting CNS infection risk in aging populations.

Importantly, the altered methylation patterns observed with advanced age are not exclusive to IL-1 $\beta$ . Recently, Yamanashi et al reported similar findings for tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ). In the brain, there appears to be a universal age-associated decrease in DNA methylation near the *TNF* gene, with this finding most pronounced in glial populations. This group mapped the methylation patterns in human subjects, discriminating between patients with and without a history of delirium, and identified certain genomic domains that become hypomethylated with aging across all individuals. However, there were more pronounced hypomethylated hotspots in delirium patients that included the *TNF* gene, suggesting that *TNF* hypomethylation may predispose aging patients to developing delirium. Interestingly, delirium is a common comorbidity of infections, especially those affecting the CNS.<sup>43-45</sup> It remains to be determined whether *TNF* hypomethylation is also a SIRT1-dependent DNMT response or if it occurs via an alternative mechanism. As more information becomes available, it will be important to consider the effects of aging and epigenetic regulation of microglial responses on CNS infection.

## 2.2 | Astrocyte epigenetics

As the most abundant glial cell population in the brain parenchyma, astrocytes serve a number of important roles in CNS homeostasis, including providing nutrients to neurons, synapse maintenance, ion and neurotransmitter regulation, as well as contributing to immunological defense.<sup>46</sup> Astrocytes are also known to interact with CNS immune cell infiltrates during disease or infection, the details of which are reviewed elsewhere.<sup>47</sup> A single astrocyte may contact as many as two million synapses and over 95% of astrocytes are estimated to contact blood vessels, placing them at a prime position to serve as sensing intermediaries between the CNS and periphery,

potentially monitoring and inducing peripheral immune cell influx into the CNS.<sup>47</sup>

Despite their central role in CNS homeostasis and disease, investigation of the epigenetic determinants that influence the immunological attributes of astrocytes is in its infancy. However, one area where significant progress has been made involves the role of epigenetics in astrocyte responses to viral infection.<sup>48</sup> Human immunodeficiency virus (HIV) is a lentivirus whose genome integrates into host DNA to establish latent infection with periods of lytic replication and expansion within the host.<sup>49</sup> While current combined antiretroviral therapy (cART) is remarkably effective at reducing viral load below the limit of detection,<sup>50</sup> occasional transient increases in HIV replication within treated individuals have been reported, indicating the existence of hidden sanctuary sites within the body that are permissive for viral replication.<sup>48</sup> Numerous groups have identified one of these sites as the CNS, with microglia and astrocytes as functional reservoirs for the virus, which is confounded by the blood-brain barrier that limits drug penetrance.<sup>48,51-53</sup> Interestingly, these transient increases in viral load due to HIV replication within astrocytes may be promoted by proinflammatory signals, as Narasipura et al<sup>48</sup> found that TNF- $\alpha$  stimulation of astrocytes increased HIV mRNA expression by twofold to sixfold and was associated with the release of infectious virions. Further, this group found that HDAC2 action and histone methylation were crucial for viral suppression, as inhibiting these pathways in astrocytes enhanced viral transcription. Accordingly, HDAC expression is known to increase under certain proinflammatory stimuli including NF- $\kappa$ B activation,<sup>54</sup> which would presumably further repress HIV transcription. These contradictory findings demonstrate the need for further work to identify proinflammatory mechanisms that favor latent or lytic HIV states within sanctuary astrocytes.

Epigenetic responses appear equally important during Zika virus infection, for which astrocytes are also a reservoir.<sup>55</sup> Maternal Zika infection is associated with fetal abnormalities including microcephaly and skull malformations. In their recent paper,<sup>56</sup> Anderson et al<sup>56</sup> assessed genome-wide methylation patterns in seropositive healthy infants, seropositive affected infants, and healthy controls. Interestingly, there was little difference in the methylation patterns of healthy individuals, regardless of seropositive status. Conversely, affected infants had substantially different methylation patterns compared to seropositive unaffected individuals. These abnormalities included hypomethylation of a set of interferon-stimulated genes (ISGs), most strongly near ISG15, which is known to inhibit Zika replication by restricting infectivity and host cell binding. Since hypomethylation would increase ISG15 transcription and restrict viral pathogenesis, it likely represents a beneficial innate adaptation to infection. This study also detected methylation changes surrounding the transcription factor Dp-1 (*TFDP1*) gene, which controls the expression of multiple genes including microcephalin 1 (*MCPH1*) that is linked to congenital microcephaly. This hints at the potential of epigenetic mechanisms to influence Zika-induced birth defects.<sup>56</sup>

In the previous section, we reviewed a role for BRD2 and BRD4 in promoting the transcription of certain proinflammatory genes in

microglia. Astrocytes have also been found to rely on these acetylation reader proteins for proper proinflammatory function. Choi et al.<sup>57,58</sup> demonstrated increased expression of the BET protein BRD2 within astrocytes in response to LPS stimulation. Specific knockdown of BRD2 in astrocytes reduced plasminogen activator inhibitor 1 (PAI-1) expression, a serine protease expressed by astrocytes in response to inflammatory challenge and dysregulated in multiple CNS diseases. Interestingly, this same knockdown strategy failed to modulate cytokine production and recapitulate the full effects of pan-BET inhibition, suggesting that each BET protein may specifically control a different aspect of the inflammatory response.<sup>58</sup> The cellular benefit of this functional segregation of BET protein responsibilities is profound, potentially tailoring induction of a particular subset of genes from the larger group of inflammation-induced acetylated genomic loci to fit the challenge at hand. This may also contribute to the heterogeneity seen within even a single population of cells during an infection response, despite similar acetylation landscapes.

In conclusion, epigenetic changes in astrocytes can alter the mechanics of viral infection, inflammatory mediator production, and may contribute to infectious complications, such as in the case of Zika virus infection. Pertinent to their immunological function, astrocytes are significant producers of chemokines within the CNS.<sup>59</sup> One such chemokine produced by astrocytes<sup>59</sup> and controlled via epigenetic mechanisms<sup>60</sup> is CCL2. CCL2 is a major chemoattractant for monocytes and macrophages into the CNS via effects at the blood–brain barrier.<sup>61</sup> CCL2 expression is regulated by the acetylation system, with increased production seen following HAT activation and subsequent histone hyperacetylation.<sup>60</sup> Additionally, histone trimethylation at H3K4 is an activating histone methylation mark and is associated with increased CCL2 production, suggesting the existence of multiple epigenetic systems regulating the production of this chemokine.

Of note, microglia also produce CCL2 along with astrocytes in response to activating stimuli. This is important as it amplifies glial-mediated recruitment of peripheral immune cells to the brain parenchyma in response to infection, which are not normally present in large numbers within the healthy CNS. We just discussed examples of the various ways that glial cells epigenetically respond to infection and regulate inflammation. As these first responders promote peripheral leukocyte recruitment, we will turn our discussion to the epigenetic factors affecting the function of these recruited leukocyte populations.

### 3 | INFILTRATING INNATE IMMUNE CELLS

#### 3.1 | Neutrophil epigenetics

Historically considered short-lived transcriptionally fixed cells,<sup>62</sup> emerging evidence suggests that neutrophils (PMNs) have a more diverse set of transcriptional programs.<sup>63</sup> PMNs are produced in greater numbers than any other immune cell in circulation with a

survival of only 12–24 h.<sup>62</sup> Importantly, healthy tissues are thought to be largely devoid of PMNs, with infiltration as a hallmark of inflammation or pathology. In the CNS, small populations of PMNs can be found in meningeal spaces,<sup>64</sup> but this is greatly overshadowed by a massive influx into the brain during inflammatory or infectious conditions.<sup>9</sup>

Mature PMNs do not replicate in response to pathogen contact, instead exiting the bone marrow with an arsenal of pre-synthesized granules that contain various classes of enzymes and antimicrobial proteins. As such, they were assumed to require little transcriptional or translational input when exerting their effector functions.<sup>62</sup> Determining the extent to which transcriptional changes occur throughout the PMN lifespan is an active field of research. Using scRNA-seq, Khojraty et al.<sup>63</sup> have identified heterogeneous PMN populations under steady-state conditions as well as during bacterial infection. To better understand these changes, this group analyzed chromatin dynamics in migrating PMNs, identifying two distinct remodeling events at the bone marrow-to-blood and blood-to-tissue transitions. During initial release from the bone marrow, PMNs undergo chromatin remodeling to favor genes associated with antigen presentation and altered metabolic activity.<sup>63</sup> Notably, PMNs are highly reliant on aerobic glycolysis<sup>62</sup> to ensure they can still function optimally in a low oxygen environment that is often encountered during inflammation.<sup>63</sup> In addition, glycolysis generates metabolic products that are critical for PMN antimicrobial activity, including NADPH via the pentose phosphate pathway that is linked with glycolysis and required for NADPH oxidase function and reactive oxygen species production. The next identified shift in chromatin accessibility in circulating PMNs occurred during transmigration into inflamed tissue, which was associated with increased accessibility and transcription of genes involved in inflammatory responses, degranulation, and adhesion.<sup>63</sup> Presumably, the activation of these genes upon tissue migration occurs to limit systemic inflammation and tissue damage at sites that do not require PMN action. Further, chromatin changes were identified near distinct sets of transcription factor loci at each PMN transition state, serving as another layer of epigenetic regulation over transcriptional programs. Interestingly, no further chromatin changes were detected following transmigration as PMNs approached the site of inflammation, despite continued changes in gene transcription. This may reflect the continued action of the altered chromatin states near transcription factors that was initiated during the blood–tissue transition.<sup>63</sup> In summary, PMNs undergo distinct epigenetic changes during at least two points in their life cycle, highlighting the rapid and dynamic nature of these modifications. By extension, this suggests that similar patterns may be important for tailoring PMN responses to CNS infection, but this remains speculative at the present time. If true, the epigenetic remodeling events corresponding to heightened inflammatory gene expression would likely occur at the blood–brain barrier. Accordingly, targeting this transition point for epigenetic evaluation of PMNs (or any infiltrating immune cell) could identify unique transcriptional programs pertinent to brain infection. Whether these changes would differ during leukocyte extravasation into the CNS vs. peripheral tissues remains to be determined.



### 3.2 | Monocyte and macrophage epigenetics

Monocytes are bone marrow-derived mononuclear phagocytes which differentiate into macrophages upon migration into peripheral tissues.<sup>35</sup> The CNS harbors several resident macrophage populations, including perivascular, meningeal, and choroid plexus macrophages. These populations are small under steady-state conditions but are capable of pronounced proliferation in response to inflammatory challenge. Additionally, during infection or pathology, circulating monocytes are recruited into the CNS where they mature into macrophages, aiding the effector responses of microglia as previously discussed,<sup>9</sup> and regulating the adaptive immune response via antigen presentation. As a prototypical innate immune cell, a great deal of research has been conducted on macrophage epigenetics, leading to elucidation of intricate regulatory mechanisms—two of which we will discuss below.

While the components of NF- $\kappa$ B signaling have been well characterized,<sup>65</sup> new epigenetic technologies are uncovering more granular details regarding the strength and temporal importance of stimuli to this vital immune pathway. The clearest example of the importance of chromatin structure to NF- $\kappa$ B activity was recently illustrated by Cheng et al.<sup>66</sup> that has helped to explain the heterogeneity of NF- $\kappa$ B action in response to different inflammatory stimuli. NF- $\kappa$ B is a prototypical transcription factor that induces the expression of many proinflammatory genes. NF- $\kappa$ B is tightly controlled through its association with I- $\kappa$ B in the cytoplasm, which sequesters NF- $\kappa$ B and prevents its downstream function. In response to an appropriate stimulus, I- $\kappa$ B is rapidly phosphorylated and degraded, allowing NF- $\kappa$ B to migrate to the nucleus. At this point, NF- $\kappa$ B binds to a sequence-specific site within the double-helix secondary structure of DNA to initiate transcription, a process that is blocked by the heterochromatin conformation of nucleosomes.<sup>67</sup> As previously discussed, this closed chromatin is maintained via attractive forces between positively charged histones and negatively charged DNA. These electrostatic interactions are relatively weak forces, allowing natural variations in molecular energy to transiently overcome the attractive forces and briefly allow transcriptional machinery access to DNA, a phenomenon termed “nucleosome breathing”.<sup>68</sup> Nuclear NF- $\kappa$ B may access promoter binding sites in target genes during these periods of relaxation, initiating gene transcription even before permissive epigenetic marks have been added in the region. Further, Cheng et al.<sup>66</sup> argued that different types of stimuli are more or less efficient at this process, owing to variable signal strength and subsequent concentrations of free NF- $\kappa$ B. Since NF- $\kappa$ B is dynamically controlled by both the strength of inflammatory signal and I- $\kappa$ B levels, the authors hypothesized that a temporal aspect of regulation exists mediated by nuclear NF- $\kappa$ B oscillations that peak during conditions of high and prolonged signaling. Indeed, NF- $\kappa$ B-mediated transcription induces I- $\kappa$ B in a negative feedback loop, which would contribute to these oscillatory levels.<sup>69</sup> This was confirmed using I- $\kappa$ B deficient macrophages that exhibited non-oscillatory signaling patterns and more robust transcriptional changes and chromatin remodeling than wildtype cells. This may represent a threshold mechanism to prevent

proinflammatory activation in response to meager stimuli, as less noxious signals would not stimulate sufficient I- $\kappa$ B degradation to allow NF- $\kappa$ B to overcome negative feedback mechanisms and reach a critical level in the nucleus. Accordingly, with strong and persistent proinflammatory signaling, sustained NF- $\kappa$ B activation would permit repeated disruption of breathing chromatin structures, recruitment of chromatin remodeling machinery, and the formation of more permanent permissive epigenetic marks.<sup>66</sup> Conceptually, this is similar to the biochemical principle of enzyme kinetics, where a particular activation energy must be reached (aka persistent NF- $\kappa$ B action) to favor progression of the reaction (aka chromatin reorganization). Importantly, this process might provide a rheostat for fine-tuning leukocyte activation via altered basal levels of NF- $\kappa$ B and I- $\kappa$ B. Given their similarities to macrophages, this process may occur in microglia as well; however, further work is needed to confirm this possibility. If true, this could represent another mechanism for the brain to establish a higher threshold for triggering inflammatory responses to protect neurons that would be easily damaged by the same level of inflammation that occurs in peripheral tissues.

The “permissive epigenetic marks” alluded to in the previous paragraph can take many forms, with acetylation being one of the best characterized in macrophages. The mechanics of this system were described earlier, with HATs and HDACs as writers and erasers of acetylation, respectively. Canonically, HDACs repress gene transcription, as they facilitate chromatin condensation. By extension, HDAC inhibition would be expected to increase transcription as HATs would act unopposed, which has been reported to occur.<sup>70-72</sup> BRD4 functions similarly in macrophages, where it has been reported to read acetylation marks near ISG elements to promote transcription. However, a recent study by Marie et al. described a mechanism where HDAC inhibition reduced ISG transcription, highlighting the complexity of ISG regulation. While total BRD4 levels in the nucleus remain constant, the amount of free or acetyl group-bound (active) BRD4 varies in response to changes in acetylation. During HDAC inhibition, unopposed HAT action led to hyperacetylation of the genome, as expected. In turn, this caused diffuse nonspecific binding of BRD4 throughout the genome, with undetectable levels of free BRD4 in the nucleoplasm. Subsequent interferon stimulation was then unable to properly induce ISG expression, as no BRD4 remained available to target transcription at these loci.<sup>17</sup> Our laboratory has observed a similar phenomenon in multiple immune cell populations, including microglia, where HDAC inhibition prior to *S. aureus* infection blocked proinflammatory cytokine production even in the face of increased HAc, mimicking BRD4 inhibition itself (unpublished data). While large-scale HDAC inhibition is unlikely to occur naturally in immune cells, various pathogens secrete epigenetically active virulence factors which co-opt host transcriptional programs to promote a more pathogen-hospitable environment.<sup>73</sup> This is a relatively recent discovery, with undoubtedly many more examples of these virulence mechanisms waiting to be identified. Given these findings, HDAC inhibition could be an attractive mode of pathogen–host genetic interference; however, this remains speculative. This is an important issue to consider in

the clinical environment, as HDAC inhibitors have been proposed for use as novel anti-inflammatory agents for chronic inflammatory and autoimmune diseases.<sup>74-76</sup> Additionally, HDAC inhibition is utilized to treat certain malignancies, an approach that markedly increases infection risk.<sup>11,13,77</sup> Therefore, sustained HDAC inhibition, either by pathogens or pharmaceuticals, may hinder the ability of the immune system to mount an effective response to infection, illustrating a potential pitfall in the clinical application of HDAC inhibitory compounds.

### 3.3 | Natural killer cell epigenetics

The final innate immune cell population we will review in terms of epigenetics is natural killer (NK) cells. NK cells originate from a lymphocytic lineage and are responsible for lysing pathogen-infected or malignant host cells.<sup>78</sup> NK cells are most closely related to CD8<sup>+</sup> T lymphocytes, although they express variable amounts of Tcell, Bcell, and myeloid signaling proteins.<sup>79</sup> Like other immune populations, NK cells migrate into the CNS during pathology, and their depletion is associated with improved neurogenesis and less cognitive dysfunction in certain neurodegenerative disorders.<sup>9</sup> NK cell activation is associated with epigenetic remodeling, and Schlums et al<sup>79</sup> demonstrated that NK cell activation during cytomegalovirus (CMV) infection was determined by altered DNA methylation patterns. Within the CNS, CMV can establish chronic infection with latent and lytic stages<sup>80</sup> and is a common cause of encephalitis in immunocompromised individuals.<sup>81,82</sup> Schlums et al<sup>79</sup> investigated NK cell heterogeneity during CMV infection and found that particular NK cell populations underwent significant expansion during viral reactivation while losing B cell and myeloid signaling molecules and adopting a Tcell signaling signature. The suppression of B cell and myeloid genes was mediated by specific hypermethylation, with hypomethylation occurring at the Tcell loci. Importantly, similar to Zika virus infection,<sup>56</sup> these epigenetic changes only occurred in individuals who were both seropositive and experienced viral resurgence, since seropositive patients with latent infection displayed normal NK cell populations. This illustrates an epigenetic mechanism by which NK cells tailor their signaling repertoire to the pathogen at hand, sparing immunological resources and tissue damage associated with more generalized immune action. As previously mentioned, CMV-associated encephalitis is most commonly observed in immunocompromised individuals, suggesting that this epigenetic mechanism may help immunocompetent hosts avoid clinical pathology.

## 4 | ADAPTIVE IMMUNE CELLS

### 4.1 | Bcell epigenetics

B cells are negligible in the CNS under normal conditions; however, their numbers increase during several neuroinflammatory diseases and infection.<sup>83</sup> The impact of B cells in the CNS

is context-dependent; on the one hand, they can exert beneficial effects by producing opsonizing antibodies and various cytokines to promote pathogen neutralization. However, during various CNS autoimmune disorders, B cells play a key pathological role due to antibody-mediated complement activation and maladaptive cytokine secretion.<sup>9</sup> Recently, Soldan et al<sup>14</sup> uncovered an epigenetic basis for Bcell infiltration of the CNS following Epstein-Barr virus (EBV) infection. EBV is a herpesvirus that causes infectious mononucleosis and mounting evidence suggests a causative link between EBV and multiple sclerosis (MS) in predisposed individuals.<sup>84-86</sup> To understand a potential mechanism for CNS disease following EBV infection of B cells, Soldan et al. performed serial transfers of an immortalized Bcell line with latent EBV infection in mice. Following each transfer, B cells that successfully migrated to the brain were recovered and adoptively transferred into the next round of animals. This process resulted in an enriched highly neuroinfiltrative Bcell population following a few transfer cycles that correlated with clear symptoms of CNS dysfunction. This population of B cells was then recovered for epigenetic and transcriptomic analysis, which identified profound transcriptional upregulation and concurrent activating histone methylation marks associated with the osteopontin (OPN) locus, among other genes. Treatment of mice with an OPN neutralizing antibody reduced Bcell neuroinvasion, improved disease course, and increased survival.<sup>14</sup> This suggests that OPN expression during EBV infection is under epigenetic control, linking a pathogen-induced epigenetic shift to increased neuroinvasion and potentially MS development. Additionally, it illustrates the power of combined transcriptomic and epigenetic sequencing techniques to identify important targets of infection. The exact mechanism of OPN involvement in Bcell neuroinvasion during EBV infection remains to be defined. However, the epigenetic correlates of this phenomenon may provide accessible targets for pharmaceutical intervention in the future, by utilizing compounds to inhibit epigenetic-mediated migration of infected B cells to the CNS and reduce the risk of subsequent MS development.

### 4.2 | Tcell epigenetics

While sparsely found in healthy brain parenchyma,<sup>47</sup> T lymphocytes represent the vast majority of immune cells in healthy CSF, most of which are of a memory phenotype.<sup>9</sup> Similar to other peripheral immune cell populations, dramatic Tcell infiltration is observed during infection and numerous CNS diseases.<sup>87</sup> Unsurprisingly, many epigenetic processes have been identified as key mechanisms dictating Tcell differentiation and fate.<sup>18,88-91</sup> Histone acetylation, histone methylation, and DNA methylation are all involved in the control of CD4 gene silencing in CD8<sup>+</sup> T cells. Histone methylation, and specifically the H3K9me2 histone methyltransferase G9a, is responsible for appropriate cytokine production in CD4<sup>+</sup> T helper cell subsets, and DNA methylation is important for memory Tcell formation.<sup>18,92,93</sup> We will explore some of these processes below.

One CD4<sup>+</sup> T cell subset with particular importance to the CNS during neuroinflammation is the T regulatory cell (T<sub>reg</sub>). T<sub>regs</sub> produce IL-10 to suppress inflammatory cytokine production by other immune populations. As such, T<sub>regs</sub> are generally regarded as neuroprotective by attenuating astroglial and neuronal death in models of neurodegenerative disease<sup>9</sup>; however, they may be counterproductive during infection. Demethylation of a region of the *FOXP3* locus is required for expression and maintenance of the T<sub>reg</sub> phenotype.<sup>85</sup> Expanding on this finding, Garg et al<sup>88</sup> identified an additional layer of epigenetic control to promote T<sub>reg</sub> programming, namely B lymphocyte-induced maturation protein-1 (BLIMP-1) regulation over DNA methylation enzymes. BLIMP-1 is a zinc finger transcriptional regulator with multiple roles in adaptive immunity, including plasma cell, CD8<sup>+</sup> T cell, CD4<sup>+</sup> T cell, and T<sub>reg</sub> differentiation. BLIMP-1 is upregulated in the inflamed CNS, where it potently inhibits Dnmt3a expression, a methyltransferase responsible for T cell programming. This effectively fixes the T cell transcriptome in a T<sub>reg</sub> state. Conversely, BLIMP-1 loss attenuated IL-10 production and increased IL-17 and IFN- $\gamma$  expression, prototypical Th<sub>17</sub> and Th<sub>1</sub> cytokines, respectively.<sup>88</sup> In another example of epigenetic determination of CD4<sup>+</sup> T cell fate, deacetylation of the *CD8* locus occurs during CD4<sup>+</sup> lineage commitment. This process is dependent on the transcription factor ThPOK,<sup>86</sup> which interacts with various corepressors and HDACs and is silenced in CD8<sup>+</sup> T cells.<sup>89</sup> Rui et al demonstrated that HDAC4 is recruited to ThPOK at the *CD8* locus, where the resulting complex deacetylates the associated histone to repress *CD8* transcription. The use of protein complexes to direct HDAC activity for site-specific action is a common mechanism used by cells to regulate epigenetic changes in lieu of non-discriminate HDAC action.

Numerous examples of CD8<sup>+</sup> T cells utilizing epigenetic mechanisms to dictate cell fate have also been described. As previously discussed, BRD4 is an acetylation reader protein that binds acetylated lysine residues and promotes molecular scaffold formation for controlled gene transcription. This is particularly important for maintaining the phenotype of CD8<sup>+</sup> effector T cells in response to infection, which is lost following BRD4 inhibition.<sup>90</sup> Further, BRD4 binding overlapped with over 99% of super-enhancers assumed to control CD8<sup>+</sup> T cell differentiation, hinting at its ability to act as a transcription factor in addition to its classical role in protein complex recruitment.<sup>90</sup> Another mechanism by which CD8<sup>+</sup> T cells epigenetically regulate effector function involves DNA methylation. Upon infection resolution, approximately 90% of CD8<sup>+</sup> effector T cells undergo apoptosis, with 10% persisting to acquire a memory phenotype.<sup>94</sup> Using deep sequencing techniques, Scharer et al<sup>91</sup> performed comparative methylation analyses on naive and effector CD8<sup>+</sup> T cells, identifying shifts in a large array of genes. As expected, increased DNA methylation corresponded with decreased gene transcription, with most changes clustering around genes known to regulate T cell differentiation. Further, many of these shifting methylation patterns occurred near transcription factor genes, adding yet another layer of complexity to cell fate determination.<sup>91</sup> The balance of T cell phenotypes during CNS infection affects the success of the immune response. We have shown that T cells selectively invade the brain

parenchyma during *S. aureus* craniotomy infection<sup>95</sup> and that CD4<sup>+</sup> T cell loss leads to increased bacterial burden (unpublished observations). The molecular mechanisms whereby CD4<sup>+</sup> T cells promote *S. aureus* containment, including epigenetic alterations, remain to be identified. Collectively, the work described above demonstrates that T cell function is achieved and maintained by epigenetic programs, allowing them to mount a successful response to infection.

## 5 | INFECTION RESOLUTION

The resolution of infection and restoration of immunological quiescence are crucial. If this process is dysregulated, either by prolonged inflammation or premature anergy, it can result in bystander tissue damage. This is especially important in the CNS, as collateral injury risks permanent damage to largely nonregenerative neural tissue. Accordingly, the study of CNS infection resolution is a field large enough for its own review, but we will touch on a few mechanisms mediated by epigenetic marks which are involved in the post-infectious processes of anergy and immunological memory.

For decades, clinicians have reported that, following the resolution of sepsis, patients exhibit increased susceptibility to a second infection.<sup>96,97</sup> This has since been linked to chronic immunological suppression, referred to as immunological exhaustion or anergy, and presumably is an adaptive mechanism to dampen the inflammatory response to the initial infection. Elements of this phenomenon are thought to have epigenetic origins,<sup>54</sup> with HDACs implicated in the induction of cellular anergy. In myeloid cells, NF- $\kappa$ B activation is associated with increased HDAC activity,<sup>98</sup> which subsequently represses many of the proinflammatory genes induced following LPS exposure.<sup>99</sup> However, the situation is likely more complex than this, as the timing of when HDAC enzymes are inhibited may yield different effects. For example, inhibiting HDAC activity prior to pathogen exposure may restrict the ability of leukocytes to remodel their epigenome to achieve optimal cellular activation, whereas blocking HDAC action after pathogen challenge could attenuate cellular exhaustion. Therefore, it is likely that HDACs exert divergent roles during activation and anergy and more work is needed to identify which complexes are involved at distinct stages throughout infection.

Epigenetics have also been implicated in establishing immunological memory. Memory responses protect against reinfection with the same pathogen, although some organisms, such as *S. aureus*, escape immune memory to cause recurrent infections.<sup>100-102</sup> An ongoing controversy in this field surrounds whether memory immune cells are derived from naive cells, or from a subset of surviving effector cells following infection. Using epigenetic techniques, Youngblood et al<sup>103</sup> uncovered evidence supporting the effector-derived memory cell model in CD8<sup>+</sup> T cells. Memory CD8<sup>+</sup> T cells are important mediators of long-term immunological memory; however, many genes associated with these memory cells are also expressed in naive CD8<sup>+</sup> T cells and suppressed in effector cells. L-selectin is an example of a gene with this expression profile, allowing naive and

memory cells to migrate to lymphoid organs and effector cells to extravasate to infected tissues. Interestingly, these expression patterns directly correlate with DNA methylation, as conditional loss of the methyltransferase Dnmt3a abolished these relationships. Although L-selectin was reduced in Dnmt3a-deficient effector cells, they regained L-selectin expression more rapidly after infection resolution than their wildtype counterparts.<sup>103</sup> This illustrates the existence of multiple layers of genetic regulation, as gene suppression can still occur in the absence of epigenetic control. Youngblood et al.<sup>103</sup> leveraged epigenetic patterns to support the concept that CD8<sup>+</sup> effector T cells can dedifferentiate into a memory population, a process regulated by the loss of epigenetic marks that were acquired during effector cell development. Modulating this process increased the efficiency of memory cell generation from CD8<sup>+</sup> effector cells, highlighting the reversibility of epigenetic marks and the utility of their plasticity.

## 6 | DISCUSSION

Current evidence suggests that epigenetics plays a pivotal role in regulating the immune response to CNS infection, modulating the activation of glia and resident macrophage populations to infiltrating innate and adaptive immune cells. With the need to rapidly respond to infectious insults, immune cells take full advantage of the speed and reversibility of most epigenetic marks. We have discussed how critical processes such as cytokine production, cell differentiation, migration, and anergy are dictated by the cooperation between transcription factors and corresponding epigenetic marks (Figure 1). We have also explored how viruses can alter these marks in ways both beneficial and detrimental to the host. Finally, we have examined how the small chemical modifications of histones by methyl and acetyl groups can have dramatic effects on cellular activation, underscoring the potency of genomic regulation. While truly fascinating work has been performed in this space (a small sample of which has been reviewed here), epigenomics is a rapidly growing field and emerging new technologies are enabling researchers to interrogate the implications of epigenetics in greater detail. We will conclude this review with a discussion of these new technologies and speculate on exciting new avenues for future exploration and clinical applications.

### 6.1 | Technological advancements in epigenetic research

The advent of single-cell sequencing technologies has revolutionized our appreciation of cellular heterogeneity, which is also applicable to epigenetic research. The Assay for Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq) was developed nearly a decade ago to identify genomic regions of open chromatin.<sup>104</sup> Shortly after, adaptations of this protocol for single-cell analysis emerged,<sup>105</sup> and the technique has been steadily refined to

improve data quality. Importantly, scATAC-seq can resolve changing chromatin organization that may be undetectable with bulk ATAC-seq technologies. As we have discussed, single-cell platforms are especially useful for studying rare cell populations, such as memory T cells, as only a small number successfully transition to a memory phenotype.

While a powerful technology, ATAC-seq does not identify the actual chemical marks that influence overall chromatin structure. Permissive methylation and acetylation marks may appear identical in ATAC-seq analysis, as this technique only assesses DNA accessibility. For years, the gold standard for localizing chemical epigenetic marks has been chromatin immunoprecipitation with parallel DNA sequencing (ChIP-seq). However, this technique is complex, time intensive, and requires a large number of cells, making ChIP-seq difficult-to-impossible to perform on rare cell populations. Similar to ATAC-seq, newer technologies based on the same principles of ChIP-seq have emerged to expand access of epigenetic investigation to laboratories without expertise in ChIP protocols. One of these new tools is Cleavage Under Targets and Release Using Nuclease (CUT&RUN) that generates ChIP-seq-like data in hours instead of days with orders of magnitude fewer cells.<sup>106</sup> Requiring no specialized skills and only common laboratory equipment, CUT&RUN can be performed on the bench top in a single tube. This is facilitated using a validated antibody targeted to an epigenetic mark of interest (such as H3K27ac). Following cell permeabilization and incubation, an endonuclease linked to staphylococcal protein A is added. Protein A directs the endonuclease to the antibody-bound epigenetic marks and facilitates DNA cleavage at these locations. The resulting DNA fragments can then be purified and sequenced. Due to the enzymatic specificity of this protocol, data generated have unparalleled signal-to-noise ratios and high concordance with ChIP-seq data while being generated at a fraction of the cost. This same process can be applied to mapping transcription factor binding sites throughout the genome with the use of an appropriately targeted antibody.<sup>107</sup> While CUT&RUN is not applicable to a single-cell platform, variants of this procedure have been developed to fill this need. Cleavage Under Targets and Tagmentation (CUT&TAG) is one example, which uses a transposase instead of an endonuclease<sup>108</sup> that provides two benefits. First, tagmentation increases the signal-to-noise ratio as the Tn5 transposase can only catalyze one reaction, eliminating repetitive DNA cleavage that can occur with the CUT&RUN endonuclease. Second, the use of a transposase has been leveraged to simultaneously complete library preparation during the experiment, eliminating the need for separate processing steps before sequencing.<sup>108</sup> Importantly, these new techniques become exponentially more powerful when coupled with parallel RNA-seq analysis, allowing direct correlation between epigenetic changes and transcriptional shifts in a single sample. While commercial kits are available for both technologies, attention must be given to the strengths and limitations of each for achieving the specific goal of an experiment. For example, CUT&RUN remains the best choice for mapping transcription factor binding due to protein size and procedural considerations,<sup>107</sup> while CUT&TAG is currently the only option for single-cell

applications.<sup>108</sup> Regardless of which technique is utilized, the similarity between ChIP-seq and CUT&RUN/CUT&TAG platforms necessitate only small adaptations to existing ChIP-seq analysis pipelines to translate existing tools for these new applications. Construction of bioinformatic pipelines for these new epigenetic tools is rapidly evolving, as most of the progress with these techniques and analysis tools have occurred in the last five years.<sup>109-111</sup> In any case, due in part to the development of these technologies, the epigeneticist's toolbox continues to rapidly expand.

## 6.2 | Immunometabolism and epigenetics

There has been an explosion of interest in the field of immunometabolism, advancing our understanding of how metabolic changes influence immune cell function. Macrophages and microglia preferentially utilize oxidative metabolism under resting conditions, shifting rapidly to aerobic glycolysis upon activation.<sup>112</sup> This transition to aerobic glycolysis involves the formation of two "breaks" in the TCA cycle that provide metabolite intermediates which influence inflammatory polarization.<sup>68</sup> In the first section of this review, we discussed the metabolic sources for various epigenetic marks and their susceptibility to nutrient availability. In the case of acetylation, mitochondrial metabolism plays a significant role in the generation of acetyl-CoA for this mark.<sup>4,9</sup> It is unknown whether the TCA cycle breaks during aerobic glycolysis cause fluctuations in metabolite levels which then feedback to alter, or even induce, the acetylation of genomic loci resulting from acetyl-CoA accumulation. This possibility is not unprecedented, as we discussed how the exotic lactylation mark is directly stimulated by lactate accumulation under inflammatory conditions.<sup>26,113</sup> Furthermore, nutrient availability in different tissue microenvironments may predispose immune cells to specific epigenetic states, priming genomic reorganization prior to the engagement of more classical proinflammatory signaling pathways following pathogen exposure. Similarly, methylation may be susceptible to changes in amino acid bioavailability and competition between the pathogen and host for these molecules. Therefore, nutrient competition could be one mechanism whereby bacteria and/or viruses exert epigenetic control over the host immune system. Answers to these complicated issues can be addressed utilizing the array of new epigenetics technologies, and no doubt will generate fascinating insights into the pathology of infection in the future.

## 6.3 | Translational epigenetics

The epigenetic mechanisms governing immunological function are diverse and present clinicians with an exciting opportunity to modulate disease progression. While the treatment of chronic disorders with some genetic basis or predisposition is complicated by the fixed nature of the genome, epigenetic marks are plastic and enzymatically mediated, making them accessible targets for clinical intervention. As is the case with most therapeutics, compounds

modulating the epigenome are likely to present as a double-edged sword with side effects that may limit their efficacy. Indeed, clinical studies investigating the efficacy of HDAC inhibitors have reported low platelet counts and reduced PMNs as adverse treatment effects.<sup>114</sup> Clearly, neutropenia may be detrimental in the case of infection; therefore, prudent clinical judgment will be paramount for the proper use of these compounds as their availability expands. Additionally, persistent HDAC inhibition is toxic to microglia, which has been leveraged to deplete microglia from mixed glial populations in vitro to yield purified astrocytes, further supporting the potential immunological danger of these compounds.<sup>115</sup> The toxic effects of HDAC inhibitors have proven beneficial in oncology, showing promise in the treatment of multiple hematologic cancers.<sup>13</sup> As of 2017, four HDAC inhibitors were approved for use by the United States Food and Drug Administration (FDA), the majority for use in the treatment of malignancy.<sup>11</sup>

While drugs that target epigenetic pathways have the potential to increase infection risk, they may still prove useful for preventing infectious complications given appropriate considerations to timing and dose. We have reviewed multiple epigenetic mechanisms that repress viral reactivation during chronic infection. It has been speculated that inhibiting the enzymes responsible for removing these suppressive marks near integrated viral DNA may be an effective strategy to maintain the clinically latent phase of some viral infections, perhaps indefinitely.<sup>116</sup> This could be useful in the treatment of a wide range of viral pathogens, including HIV, CMV, human papilloma virus (HPV), Zika virus, and EBV. While clear rationales exist for the epigenetic treatment of viral infection, the use of these methods for bacterial infection is considerably less studied and potential considerations fall in two distinct categories: (1) preventing bacterial interference of epigenetic remodeling and (2) epigenetic supercharging of the immune response. In the case of the former, a growing list of pathogens have been found to induce detrimental epigenetic changes in the host. For example, the *Mycobacterium tuberculosis* Rv1998 antigen has methyltransferase activity that suppresses inflammatory gene expression.<sup>117</sup> In this case, inhibiting epigenetically active bacterial enzyme(s) may be a useful adjunct to antibiotics, especially as antimicrobial resistance continues to be a threat. The other method for epigenetic treatment of bacterial infection involves boosting host immune function. As previously discussed, epigenetic changes are used to dictate cellular differentiation of several immune populations. With a greater appreciation for how these marks regulate cell type-specific development, epigenetic compounds could be targeted to precursor lineages via nanoparticle-based approaches to promote rapid differentiation into terminal effector cells. As an example, our laboratory studies granulocytic myeloid-derived suppressor cells (G-MDSCs), a developmentally stunted PMN-like population with significant immunosuppressive properties. First described in the context of cancer,<sup>118</sup> we have identified a detrimental role for G-MDSCs in promoting *S. aureus* prosthetic joint infection.<sup>119</sup> G-MDSC infiltrates are also present during *S. aureus* craniotomy infection and inhibit PMN killing of *S. aureus*.<sup>95</sup> Given that epigenetic changes influence leukocyte activation, and

the previously reviewed evidence for epigenetic changes in PMNs during tissue migration,<sup>63</sup> it may be possible to drive G-MDSC maturation, or any developmentally maladapted immune cell, into their optimal antimicrobial state. As previously discussed, HDAC inhibition can interfere with cytokine production by multiple innate immune cell types, suggesting that inhibition of HATs may augment this response to promote pathogen clearance. However, the relationship between epigenetics and cytokine production is likely much more nuanced in practice. Regardless, targeted drug delivery will be a key principle in designing brain permeable epigenetic therapies, as off-target effects may be especially damaging to delicate brain tissue. Finally, there are clinical scenarios where immunosuppression is paradoxically beneficial during infection. The most recent example is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) where disease severity and mortality have been linked to excessive proinflammatory cytokine production.<sup>120</sup> Currently, there are multiple ongoing clinical trials assessing the efficacy of TNF- $\alpha$  inhibitors on SARS-CoV-2 progression with promising preliminary results.<sup>120</sup> It may be possible to leverage HDAC inhibitors to control the cytokine storm in these cases, thwarting excessive cytokine release at the DNA instead of protein level.

Finally, epigenetic mechanisms may influence immune dysfunction seen in patients with metabolic disorders given the intimate relationship between epigenetics, the immune system, and metabolism. For example, obesity is associated with increased susceptibility to infection<sup>121,122</sup> and the metabolic abnormalities associated with obesity may limit epigenetic remodeling of immune cells to fight infection since certain metabolites are essential for epigenetic mark placement. Another example is diabetes, where alterations in glucose metabolism and TCA cycle activity likely influence the availability of raw materials for creating acetylation marks. These areas are ripe for future study and will benefit from the increasing availability of sophisticated epigenetic techniques.

## 7 | CONCLUSION

In the short half-century since the initial discovery of epigenetic marks, the extent to which we have learned how they govern various life processes is profound.<sup>123</sup> The intersection between epigenetics and immunology affects a wide range of processes, including inflammation, cell development, pathogen defense, and metabolism. As a rapidly expanding field with technology improving at pace to match, the study of shifting epigenetic marks holds great promise in explaining how the immune system can modulate such a diverse array of biological processes while operating with a restricted number of effector cells. We have reviewed the life cycle, mechanics, and function of multiple DNA and histone chemical marks. We have also examined the relevance of these changes to the immune attributes of both CNS resident and infiltrating immune cells and explored the expanding techniques and applications for how epigenetics can be applied to address immunological questions. Epigenetic marks are rapid, reversible, and

richly interconnected with most cellular functions. Because of this, investigation of these marks may provide the field of infectious diseases with not only interesting explanations for biological functions, but accessible and novel targets for immunomodulatory therapy. Further, as we begin to understand the various epigenetic protein complexes that regulate the precision of mark placement, we may gain the capability to design complexes with targeted genetic action, selectively and reversibly controlling gene expression at will. Indeed, preliminary in vivo work in a similar vein has been conducted, combining clustered regularly interspaced short palindromic repeats (CRISPR) technology with epigenetically active enzymes.<sup>124</sup> The scientific and clinical applications of these technologies are exciting, cementing epigenetics as an important field and vital consideration in the study of CNS infection.

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### CONFLICT OF INTERESTS

The authors have no conflicts of interest to report.

### DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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