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Histone methyltransferase and histone methylation in inflammatory T-cell responses

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Abstract

During immune responses, T cells require tightly controlled expression of transcriptional programs to regulate the balance between beneficial and harmful immunity. These transcriptional programs are critical for the lineage specification of effector T cells, the production of effector cytokines and molecules, and the development and maintenance of memory T cells. An emerging theme is that post-translational modification of histones by methylation plays an important role in orchestrating the expression of transcriptional programs in T cells. In this article, we provide a broad overview of histone methylation signatures for effector molecules and transcription factors in T cells, and the functional importance of histone methyltransferases in regulating T-cell immune responses.

Keywords

differentiation; effector T cell; epigenetic effects; histone methyltransferase; memory cell maintenance

T-cell-mediated immunity is a double-edged sword. It provides protection against infections and tumor cells, but can also cause inflammatory disorders, such as chronic infections, autoimmune diseases and rejection of allogeneic grafts after organ transplantation [1–10]. Upon antigen activation, naive CD4⁺ T cells differentiate into distinct subsets of effector cells producing lineage-specific cytokines [11–13]. Th1 CD4⁺ T cells are characterized by production of IFN- γ , whereas Th2 cells secrete IL-4, IL-5 and IL-13 [11–13]. Th17 cells are characterized by their capacity to produce high amounts of IL-17 and IL-21 [11,14]. Antigen activation of naive CD8⁺ T cells leads to generation of cytotoxic T lymphocytes that produce high levels of IFN- γ and several other cytotoxic molecules such as GZMB, PRF1 and Fas ligand [6, 8]. Impaired production of effector CD4⁺ and CD8⁺ T cells is potentially disastrous to hosts with chronic infections and tumors. By contrast, skewed and/or persistent effector responses can be detrimental to the host in terms of mediating tissue injury and lethal complications [1–10]. Thus, T cells require tightly controlled expression of transcriptional programs to regulate the balance of beneficial versus harmful immunity.

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Post-translational modifications of histones play critical roles in controlling gene transcription [15, 16]. Histones are small basic proteins that can package DNA into structural units called nucleosomes. The N-terminal tails of the core histones (H2A, H2B, H3 and H4) are subject to post-transcriptional modifications by specific enzymes that write a histone code by adding or removing chemical groups, such as acetyl, phosphoryl and methyl groups [15, 16]. For example, the acetylation of key lysine residues of histone H3 and H4 by histone acetyltransferases is known to be important for transcriptional activation, whereas the removal of the acetyl groups by histone deacetylases is shown to be associated with transcriptionally inactive chromatin [15–18]. Regulatory proteins, which possess the ability to selectively bind certain histone modifications with very high affinity, can ‘read’ the histone code to initiate programs of gene expression [15,16,18]. Collectively, modifications of histones may result in heritable changes in gene expression without altering the sequence in DNA, which are termed epigenetic effects [15,16,18].

Histone methylation is considered to be crucially involved in establishing cell type-specific gene-expression patterns [19–21]. It can occur at lysine residues, which may be monomethylated, dimethylated (me₂) or trimethylated (me₃). Depending on the site and degree of methylation, histone methylation can be either repressive (e.g., H3K9me₃, H3K27me₃ and H3K79me₃) or active (e.g., H3K4me₃, H3K36me₃ and H4K20me₃). Many studies have reported the presence of differential histone methylation marks on lineage-specific effector genes [22–32]. The *IFNG* gene in Th1 cells and the *IL4* gene in Th2 cells were marked with permissive H3K4me₃ modifications, whereas silenced lineage-promiscuous genes, such as the *IL4* gene in Th1 cells and the *IFNG* gene in Th2 cells, were marked with repressive H3K27me₃ [31, 33]. Histone lysine methyltransferases catalyze the addition of methyl groups donated from S-adenosylmethionine to histones [19–21, 34], whereas histone demethylases reduce lysine methylation by removing methyl groups from histones [19–21,34]. This review will discuss the correlative nature of histone methylation signatures for genes associated with the development of effector and memory T cells, and highlight exciting findings from recent functional studies of histone lysine methyltransferases in antigen-driven T-cell responses [25,35–40]. Histone methylation can also occur on other basic residues such as arginine, which are catalyzed by a family of arginine methyltransferases. The role of arginine methylation and its related methyltransferases in T cells remain largely unknown and are not discussed here.

Histone methylation landscape in CD4⁺ T cells

Studies on the biological roles of histone methylation have mainly focused on their correlations with the regulation of gene expression in both CD4⁺ T cells and CD8⁺ T cells, including H3K4me₃, H3K9me₂, H3K9me₃ and H3K27me₃ [26,31,33,41–43]. Data from these studies indicate that there are dynamic and complex changes in sites and degrees of histone methylation on the regulatory regions of gene loci in T cells during their differentiation to distinct subsets of effector T cells (Figure 1).

Histone methylation marks for effector cytokines

Naive CD4⁺ T cells are characterized by histone methylation signatures consistent with the quiescent state (Figure 1) [26,31,32,44]. In these cells, moderate levels of repressive H3K27me₃ were present at the 3′-UTR region of the *IFNG* gene and the intergenic region of the *IL4* gene [26,31,32,44]. H3K9me₃ and H3K4me₃ were not detected at the promoter of *IFNG* and *IL4* loci [26,31,32,44]. Notably, neither H3K27me₃ nor H3K4me₃ was detected at the promoter region of the *IL17* gene [31].

Effector differentiation triggers a dynamic change in expression of repressive versus active histone methylation marks (Figure 1). Upon Th1-cell differentiation, the regulatory region of

IFNG showed a reduction of H3K27me3, but an increase in expression of both the active mark H3K4me3 [31] and repressive mark H3K9me2 [26]. In Th1 cells, H3K27me3 extensively marked the promoter and 3'-UTR regions of *IL4* and *IL17* loci, whereas H3K4me3 was not detectable in these regions [31]. Histone methylation signatures in Th2 cells are also consistent with their phenotype of activated *IL4* transcription, but repressed the expression of *IFNG* and *IL17* genes. Th2 cells showed a reduction of H3K27me3 and a strong induction of H3K4me3 in the *IL4* locus. H3K27me3 was evident in the *IFNG* and *IL17* loci [31, 42]. Furthermore, H3K9me2 was induced in the *IFNG* locus during early Th2 differentiation [26]. Some studies examined the histone methylation marks in both mouse and human Th17 cells [31,42,43,45]. Upon differentiation into Th17 cells, H3K4me3 was evident at the *IL17A* promoter [43], whereas H3K27me3 was abundantly expressed at the *IFNG* and *IL4* loci [31]. These dynamic changes suggest a complex effect of histone methyltransferases on regulating differentiation of distinct lineages of effector CD4⁺ T cells.

Histone methylation marks for master transcription factors in Th1, Th2 & Th17 cells

Lineage-specific transcription programs have been shown to induce different subsets of effector CD4⁺ T cells [11, 46]. IL-12 activation of STAT4 and IL-4 activation of STAT6 promote Th1- and Th2-cell differentiation, respectively [11,13,14]. Th17-cell differentiation involves activation of STAT3 and a complex effect of TGF- β 1, IL-6, IL-21 and IL-23 [11,47,48]. Master transcription factors that regulate differentiation of distinct lineages have been identified. T-cell-specific T-bet (encoded by *TBX21*) is a key factor for Th1-cell differentiation, GATA3 for Th2 cells and ROR- γ t for Th17 cells [11,13,14].

Our understanding of the histone methylation landscape for transcription factors largely derives from genome-wide mapping [31, 32, 44]. H3K 27me3 strongly marked the promoter, intergenic and 3'-UTR regions of *TBX21* in naive CD4⁺ T cells and non-Th1 cells. Notably, H3K4me3 was also evident at the *TBX21* promoter in these cells [31]. Differentiated Th1 cells had reduced H3K27me3 and increased H3K4me3 at the *TBX21* regulatory regions [31]. Similar to *TBX21*, the promoter and transcribed regions of the *GATA3* gene were marked by abundant H3K27me3 and low-level H3K4me3 in naive and non-Th2 cells [31]. Upon Th2-cell differentiation, there was a significant reduction of H3K27me3 and an increase of H3K4me3 at the *GATA3* regulatory regions [31]. These dynamic changes in expression of histone methylation marks during development of Th1 and Th2 cells are consistent with activation of *TBX21* and *GATA3* transcription, respectively. It has been reported that genes with bivalent histone modifications play important roles in embryonic stem cell development [49]. Bivalent chromatin states may provide another layer of flexibility in the rapid increase of gene expression. For example, Araki and colleagues identified many bivalent genes that were associated with high amounts of H3K4me3 and H3K27me3 in resting memory CD8⁺ T cells, including genes associated with self-renewal and differentiation [33]. However, whether the colocalization of repressive H3K27me3 and active H3K4me3 at the regulatory regions of the *TBX21* and *GATA3* loci could reflect a certain degree of plasticity for developed effector T cells has yet to be formally determined.

Interestingly, the bivalent colocalization of H3K27me3 and H3K4me3 was not observed in the *RORC* gene (which encodes ROR- γ t) in naive CD4⁺ T cells and non-Th17 cells [31,45]. In these T-cell subsets, the *RORC* gene had extensive H3K27me3 marks [31, 45]. The differentiation into Th17 cells caused upregulation of H3K4me3 in the *RORC* promoter while decreasing H3K27me3. By contrast, Th1-cell differentiation resulted in upregulation of H3K27me3 within regulatory regions of *RORC* [31]. This epigenetic landscape for the *RORC* gene in Th17 and Th1 cells may explain the tight control of Th17-cell differentiation and reciprocal conversion between these two subsets in response to environmental stimuli

[11,13]; for example, IL-12 activation of T-bet led to significantly increased amounts of H3K27me3 at the regulatory regions of the *RORC* locus in Th17 cells and decreased production of IL-17 [43]. However, even in the absence of STAT4 and T-bet, which are critical transcription factors for Th1 cells, Th17 cells still required TGF- β 1 for induction of Th17 cells [50, 51]. Th17 cells are known to be potentially harmful to the host [47, 52]. Stringent control of the *RORC* gene transcription through histone methylation may represent an important mechanism to prevent the development of Th17 cell-mediated inflammatory responses [43,50,51].

Several other transcription factors, such as *Zbtb7b* (also called Th-POK), *Runx3* and the Notch effector *RBP-J/CSL*, may contribute to the development of distinct lineages of effector CD4⁺ T cells [12,14,53,54]. Some studies revealed a dynamic change in histone methylation landscape at the *STAT4* promoter [31]. Further characterization of histone methylation signatures for these transcription factors may provide guidelines for better understanding the functional role of epigenetic mechanisms in T cells.

Histone methylation signatures in CD8⁺ T cells

A prominent property of effector CD8⁺ T cells is their capacity to produce effector cytokines (TNF- α and IFN- γ) and cytotoxic molecules (GZMB, PRF1 and Fas ligand) [6,8]. Naive CD8⁺ T cells had abundant H3K27me3, but low levels of H3K4me3 at the promoter of the *IFNG* locus [41], and high expression of H3K27me3 in *GZMB* loci [55]. By contrast, they showed low amounts of H3K27me3 and high levels of H3K4me3 at the proximal promoter of *TNFA* [41]. These observations may explain why naive CD8⁺ T cells are able to produce TNF- α immediately following antigenic activation, but require several rounds of cell division to secrete IFN- γ [41]. However, how this quiescent histone methylation state in naive CD8⁺ T cells is maintained remains unclear.

Upon differentiation into effector CD8⁺ T cells, the *IFNG* promoter showed a reduction in H3K27me3, but markedly enriched H3K4me3, a signature of permissive transcription [41,55]. The *GZMB* locus, particularly in transcribed regions, was strongly marked with H3K4me3 and H3K9me3 [30,33]. Interestingly, in naive CD8⁺ T cells, H3K27me3 was lower near the transcription starting site of the *PRF1* gene compared with other downstream regions [55]. Differentiation into effector and memory cells did not result in further alteration of the repressive mark of H3K27me3 at the promoter of *PRF1*, which is not consistent with activation of *PRF1* transcription in effector CD8⁺ T cells [55]. Therefore, in effector CD8⁺ T cells, there is a distinct histone methylation signature for different effector molecules. Whether this suggests the existence of differential epigenetic processes in regulating effector molecules has yet to be defined.

Several transcription factors are found to be important for regulating effector differentiation and memory development; for example, T-bet and Eomes are known to be important for promoting effector differentiation of activated CD8⁺ T cells [56–58]. During acute immune responses, effector CD8⁺ T cells expressing high levels of T-bet were prone to terminal differentiation [59]. By contrast, during chronic infections, effector CD8⁺ T cells expressing high levels of Eomes were susceptible to exhaustion [60]. Blimp-1, which is a transcription repressor with multiple roles in promoting differentiation of activated B and T cells, promotes terminal differentiation of activated CD8⁺ T cells and induces exhaustion of chronically activated CD8⁺ T cells [61–64]. Id2, which is a member of the inhibitor of DNA-binding family, is required for the survival of effector CD8⁺ T cells [65].

Genome-wide mapping analysis began revealing histone methylation signatures for these transcription factors in CD8⁺ T cells [33]; for example, naive CD8⁺ T cells had high

amounts of H3K27me3 at the regulatory region of the *PRDM1* gene (which encodes Blimp-1), whereas memory CD8⁺ T cells showed high levels of H3K4me3, but low expression of H3K27me3 at the *PRDM1* locus [33]. This conversion from the repressive to permissive state is in agreement with progressive upregulation of *PRDM1* when naive CD8⁺ T cells differentiate to effector and memory cells [61–64,66]. Similar changes in histone methylation marks for the *EOMES* gene were observed in CD8⁺ T cells during their differentiation [61–64,66]. Further determination of the correlation between histone methylation marks and expression of transcription factor genes will provide insights into how histone methyltransferases functionally regulate these transcriptional factors.

Function of histone methyltransferases in effector T cells

Histone methyltransferases that methylate lysines of histones can be classified into three families: the SET domain-containing proteins, DOT1-like proteins and non-SET domain-containing proteins (Table 1) [67–70]. Interestingly, most of these methyltransferases typically function in large complexes formed with other proteins. For example, trithorax group (TrxG) proteins and polycomb-group (PcG) proteins are important regulators of numerous genes controlling cell proliferation, differentiation and survival [71,72]. TrxG and PcG protein complexes are commonly linked to gene activation and silencing, respectively [71,72]. The histone methyltransferase MLL is a key component of the TrxG protein complex that mediates H3K4me3 [71,73]. Ezh2 is a PcG protein that catalyzes H3K27me3 [72,74–76]. PcG proteins form two distinct macromolecular complexes called polycomb repressive complex (PRC)1 and PRC2. Major subunits of PRC2 are Ezh2, Suz12 and Eed, whereas PRC1 is composed of more diverse components, such as Bmi1, Rign, HPH and CBX molecules. Loss of any key component of PRC2 leads to disruption of PRC2 and the enzymatic activity of Ezh2 [72,77,78]. Suv39h histone methyltransferases govern H3K9 methylation at pericentric heterochromatin [67]. The selective methylation of H3K9 creates a high affinity-binding site for the heterochromatic HP1 proteins [67,79, 80]. The SUV39H1–HP1 methylation system plays important roles in chromosome segregation and gene expression [67,79, 80]. Thus, when considering the action of methyl - modifying enzymes, it is important to bear in mind that the enzymatic activities of these proteins may be regulated not only by their expression, but also by their interacting molecules. With the available genetic approaches, several groups have determined the functional relevance of these histone methyl-transferases and histone methylation pathways in T cells.

G9a

G9a is a histone methyltransferase that catalyzes H3K9me2 and mediates efficient repression of developmentally regulated genes in embryonic stem cells (ESCs) [81,82]. Chromatin immunoprecipitation (ChIP) analysis revealed that loss of G9a led to decreased levels of H3K9me2 at the *IFNG*, *IL13/IL4* and *IL17A* gene loci in CD4⁺ T cells cultured under neutral, Th1 and Th2 cell conditions [35]. Thus, G9a is responsible for the majority of H3K9me2 at these gene loci. Functional assessment using G9a conditional knockout mice showed that G9a repressed production of IFN- γ and IL-17a in CD4⁺ T cells cultured under neutral but not Th1 conditions [35]. By contrast, G9a was found to promote production of Th2 cytokines (IL-4, IL-5 and IL-13) in CD4⁺ T cells cultured under either neutral or Th2 conditions [35]. Notably, this G9a deficiency-mediated impairment in Th2 cytokine induction could not be attributed to altered levels of the Th1-associated factors, STAT4, and T-bet or Th2-associated factors, STAT6 and GATA3 [35]. Thus, as a transcription repressor, G9a exhibited both inhibitory and activating effects on effector differentiation of activated CD4⁺ T cells [35].

These G9a functions appeared to be dependent on cytokine signals received by CD4⁺ T cells. Loss of G9a did not significantly affect the production of IFN- γ and IL-17a in CD4⁺ T

cells cultured under Th1 conditions [35]. Furthermore, the absence of G9a and reduction of H3K9me2 did not cause inappropriate *IFNG* transcription in CD4⁺ T cells cultured under Th2 conditions [35]. Interestingly, studies from a different group suggested that during Th2-cell differentiation, there was a dynamic change in expression of H3K9me2 and H3K9me3 in the regulatory region of the *IFNG* locus [26]. All these observations suggest that the expression of these repressive marks across the *IFNG* locus in Th2 cells may be not required to silence *IFNG*. An alternative explanation is that other epigenetic modifications might play a redundant role in repressing *IFNG* transcription in Th2 cells. However, since the activating effect of G9a on Th2 cytokine production was independent of its histone methyltransferase activity [35], it is possible that G9a may regulate Th2-cell differentiation through a mechanism without catalyzing histone methylation.

SUV39H1

The histone methyltransferase SUV39H1 catalyzes H3K9me3 [79,80]. Upon differentiation into Th1 and Th2 cells during a primary immune response, H3K9me3 is increased at the promoters of *IFNG* and *IL4* genes in the opposite lineages [26]. Similarly, an increase of H3K9me3 was observed in the promoter of the *TBX21* and *GATA3* gene in Th2 and Th1 cells, respectively. These results appear to be consistent with a repressive effect of SUV39H1 on Th2 cytokines in Th1 cells and on *IFNG* transcription in Th2 cells. However, loss of SUV39H1 did not affect the generation of either Th1 cells or Th2 cells and the levels of H3K9me3 at these gene loci during primary responses [39].

Commitment to a single lineage requires heritable alterations to the cytokine genes themselves and/or to the lineage-specifying transcription factors during proliferation of antigen-activated T cells [11,13,14,46]; for example, earlier studies suggested that long-term stimulation significantly reduced the plasticity of Th1 and Th2 cells to differentiate into the opposing lineage [83,84]. Later studies indicated that DNA demethylation was associated with induction of high levels of IL-4 transcripts in developed Th2 cells [85]. Thus, epigenetic modifications responsible for gene silencing may help to restrict cytokine production to the appropriate lineages, which can be passed on to progeny T cells through multiple cell divisions.

An elegant study by Allan and colleagues recently indicated that SUV39H1 was essential for maintaining the stability and plasticity of differentiated Th2 cells [39]. Upon exposure to Th1-polarizing conditions, while wild-type (WT) Th2 cells retained their property to produce Th2 cytokines, SUV39H1-deficient Th2 cells were converted into Th1 cells producing IFN- γ and cells producing both IL-4 and IFN- γ [39]. This effect was associated with increased transcription of both T-bet and IFN- γ without reducing GATA3 [39]. Moreover, the frequency of GATA3- and T-bet-double positive CD4⁺ T cells was markedly increased in SUV39H1-deficient Th2 cells cultured under Th1 conditions [39]. By contrast, SUV39H1 deficiency did not affect the stability of differentiated Th1 cells under Th2-skewing conditions. Interestingly, there was no difference in H3K9me3 expression at the promoter of *TBX21* between WT and SUV39H1-deficient Th2 cells [39]. These observations identify that SUV39H1 is critical for maintaining the stability of differentiated Th2 cells via a mechanism without altering H3K9me3.

MLL

The MLL protein belongs to the TrxG protein family and specifically catalyzes H3K4me3, which is associated with permissive transcription [86]. The *MLL* transcript was strongly expressed in naive CD4⁺ T cells, but markedly decreased when they differentiated into Th1 cells and Th2 cells [25]. The levels of *MLL* mRNA increased again in memory CD4⁺ T cells, especially in Th2 cells [25]. This indicates that the expression of MLL is linked to a

quiescent state of resting T cells. It has been shown that like WT CD4⁺ T cells, MLL-haploinsufficient (MLL^{+/-}) CD4⁺ T cells differentiated into both Th1 cells and Th2 cells during primary immune responses [25]. However, MLL^{+/-} memory Th2 cells had a significantly reduced capacity to produce IL-4 compared with their WT counterparts. By contrast, MLL^{+/-} memory Th1 cells produced an equal amount of IFN- γ when compared with WT Th1 cells [25]. This impaired stability to produce Th2 cytokines was associated with significantly decreased H3K4me3 at the Th2 cytokine gene loci [25]. Thus, loss of one allele in MLL does not affect the production of Th1 and Th2 effector CD4⁺ T cells, but impairs the ability of memory Th2 cells to produce Th2 cytokines [25]. Recent studies have demonstrated that MLL involves a complex mechanism to regulate the development and maintenance of memory Th2 cells [36, 37], which is discussed below. However, whether MLL may influence the stability of differentiated Th2 cells (e.g., conversion into Th1 cells) has not been reported.

Ezh2

The histone methyltransferase Ezh2 specifically catalyzes H3K27me3 [72, 74–76]. Ezh2 forms PRC2 together with other PcG proteins, Suz12 and Eed. PRC2 is crucial for maintaining the cellular memory and transcriptional patterns [71,87,88]. Loss of Ezh2 from the double-positive thymocyte stage onwards did not impair T-cell development or homeostasis of mature T cells in peripheral lymphoid tissues [89]. Mature naive T cells expressed low levels of Ezh2. Upon T-cell receptor (TCR) ligation and alloantigen stimulation, both CD8⁺ and CD4⁺ T cells rapidly upregulated Ezh2 expression [40,89].

Some studies suggested that Ezh2 was correlated with Th1 and Th2 cell development [24,90,91]. Ezh2 bound to the *IFNG* promoter and proximal transcribed region both in Th1 and Th2 cells [91]. Notably, in developing Th2 cells, Ezh2 was also found to be associated with the *IL4* promoter [24,27]. This correlation of Ezh2 with Th1 and Th2 development might be explained by its effect on repressing transcription of the opposite cytokine gene loci. For instance, STAT6 and GATA3 have been shown to bind to the *IFNG* regulatory regions in Th2 cells. STAT6-deficient Th2 cells lacked detectable H3K27me3 across the *IFNG* locus and produced higher levels of IFN- γ than their WT counterparts [26]. On the other hand, ectopic expression of GATA3 recruited Ezh2 to the *IFNG* promoter region in Th2 cells [26]. However, whether genetic inactivation of Ezh2 leads to inappropriate differentiation of Th1 and Th2 has not been effectively tested. A recent study has shown that Ezh2 also positively correlated to the expression of *IL17A* and *IL17F* [90]. These studies pointed to a potential impact of Ezh2 and repressive H3K27me3 in multiple lineages of effector T cells.

Histone methyltransferases & maintenance of effector function in memory cells

Upon antigenic stimulation, naive T cells undergo a characteristic sequence of clonal expansion, apoptotic contraction and memory cell development. During the initial clonal expansion phase, antigen-activated T cells proliferate and differentiate into effector cells, armed to eliminate the target such as pathogens [6,8,92,93]. After clearance of the target antigen, most effector CD8⁺ T cells die, with a small fraction of antigen-experienced T cells surviving and differentiating into memory cells [59, 93–95]. Memory T cells can survive the lifetime of an individual through a mechanism of self-renewal. Upon re-encounter of the specific antigen, memory T cells can rapidly elaborate effector functions, thereby providing protection against secondary infections [8,10,93,96–99].

Active chromatin marks at effector gene loci

Recent studies suggested that the capacity of resting memory T cells to rapidly elaborate effector function could be attributed to permissive chromatin states at relevant gene loci [13,25,30,33,37,55,92,100]; for example, resting memory CD8⁺ T cells exhibit an open chromatin structure characterized by loss of the repressive mark H3K27me₃, enrichment of the active mark H3K4me₃ and increase of permissive acetylation of lysine 9 at histone H3 (H3K9ac) at the effector gene loci (e.g., *TNFA*, *IFNG*, *GZMB*, and *PRFI*) [28,30,41,55]. This permissive chromatin feature may explain why these effector genes were upregulated in resting memory T cells relative to naive T cells and rapidly increased upon TCR ligation [8,28,30,41,55,92,100,101]. Similar histone signatures have been observed for memory Th2 CD4⁺ T cells where hyper-H3K9ac and -H3K4me₃ at the Th2 cytokine gene loci were preserved in the absence of IL-4 [25, 37]. However, this histone signature may not explain why resting memory T cells can restrain the transcription of these effector genes at certain low levels in the absence of antigen. Functional studies are needed to assess if there might be a mechanism capable of actively repressing transcription of these effector genes in resting memory cells, and/or a mechanism capable of rapidly recruiting transcription factors to activate these genes upon antigenic rechallenge.

Histone methyltransferase regulation of transcription factors

Emerging evidence indicates that histone methyltransferase is important for regulating gene expression in memory cells; for example, memory Th2 cells required MLL to maintain the expression of GATA3 after clearance of the antigen. Genetic inactivation of MLL led to reduction of GATA3 expression in memory Th2 cells and their impaired IL-4 production [25, 37]. One possible explanation for maintaining the permissive histone methylation signature in memory Th2 cells is that MLL catalyzes H3K4me₃ upstream of the *GATA3* gene, leading to persistent expression of *GATA3* and active *IL4* transcription [25,37]. This process occurred independent of IL-4 and STAT6 during the memory phase [37]. Recent studies have shown that recruitment of menin and c-Myb is important for the establishment and maintenance of effector function in memory Th2 cells in the absence of IL-4 and STAT6 [37, 38]. Menin itself does not have methyl-transferase activity, but possesses DNA-binding properties. Menin, MLL and other proteins form a complex, binding upstream of the *GATA3* gene locus. Loss of menin did not affect the initial production of effector Th2 cells, but caused impaired memory Th2 cells due to decreased expression of GATA3 [37]. Another explanation is that memory Th2 cells also developed a mechanism to prevent the reassociation of Ezh2 with the upstream region of the *GATA3* gene, thereby preventing the occurrence of the repressive mark H3K27me₃ [37]. These studies indicate that the cooperation between histone methyltransferases and transcription factors is required for maintaining active chromatin states in memory cells. It will be intriguing to determine if a similar mechanism may be engaged in memory Th1 cells and memory Th17 cells.

Memory CD8⁺ T cells can be broadly categorized into two subsets: central memory T cells (T_{cm}) and effector memory T cells (T_{em}) [10,95,98,102,103]. Genome-wide studies revealed that compared with T_{cm} cells, T_{em} cells expressed more genes associated with effector function, proapoptotic signaling and certain chemokines [33,100,104]. This is in agreement with the difference in effector function between T_{cm} and T_{em}; the former are less differentiated cells lacking immediate effector function, and the latter are further differentiated cells elaborating immediate effector function. Interestingly, both subsets exhibit increased expression of common activation genes related to effector differentiation and function. These include *PRDM1*, *ID2* and *KLRG1* (which distinguish short-lived from long-lived memory precursor effector cells) [33]. These genes expressed a typical permissive epigenetic mark, that is, increased amounts of H3K4me₃, but reduced levels of H3K27me₃ at their regulatory regions. However, which histone methyltransferase(s) and

how they contribute to the maintenance of effective functions in memory CD8⁺ T cells are not clear.

Linking effector function to survival capability

Intriguingly, the histone methylation pathways required for maintaining effector functions in memory T cells are also implicated in promoting the survival of memory T cells [22,23,25,29,105]; for example, several PcG proteins such as Bmi-1, Mel-18 and Ring1A have been found to be important for promoting memory cell survival. These PcG proteins themselves do not have histone methylation enzyme activity, but can be recruited to the chromatin expressing H3K27me3 [72,90,106–108]. Loss of Bmi-1 led to reduced expression of H3K27me3 and DNA methylation at the regulatory region of *NOXA* locus (which encodes a proapoptotic molecule) and impaired survival of memory T cells and their production of IL-4 [29]. Other studies discovered that MLL, Mel-18 and Ring1B have been shown to be required for survival and effector function of memory Th2 cells [22,23,25,105]. This close link between effector function and survival capability in memory cells supports the notion that histone methyltransferases and the resulting regulation of histone methylation pathways may orchestrate a group of transcriptional programs important for memory cell development and maintenance.

PcG proteins have been found to be associated with DNA methyltransferases that methyl-ate DNA [29,77,109–111]. DNA methylation represents another important epigenetic mechanism regulating transcription activities [112]. The association of DNA methylation with the development and maintenance of memory T cells has been well reviewed [14,113,114]. In this review, the impact of DNA methylation in memory T cells will not be further discussed.

Converting extrinsic signals to T-cell-intrinsic programs

Many studies have demonstrated that the fate of antigen-activated T cells to develop into effector and memory T cells has been programmed early during the antigenic priming phase [6,8,10,97]. Emerging data indicate that histone methyltransferases may contribute to the establishment of this developmental program in antigen-activated T cells (Figure 2).

Setting up histone methylation pathways for lineage-specific genes

Recent studies have investigated the role of histone methyltransferases in setting up the transcriptional programs required to control the lineage development of effector T cells. Naive CD4⁺ T cells, which expressed low levels of GATA3, PcG proteins (Ezh2, Suz12 and Bmi-1) and H3K27me3 marks, were enriched at the upstream region of the *GATA3* gene proximal promoter [37]. Upon Th2-cell differentiation, activation of STAT6 led to dissociation of PcG proteins from the upstream region of the *GATA3* gene in differentiating Th2 cells (Figure 2). This process was dependent on the presence of functional STAT6 [37]. Dissociation of PcG proteins was accompanied with the association of MLL and Menin with the upstream region of the *GATA3* gene [37]. When MLL and Menin were recruited to sustain GATA3 expression in developed Th2 cells, STAT6 was no longer required and reassociation of PcG proteins to the upstream of *GATA3* did not occur, even in the absence of STAT6 [37]. However, loss of *MLL* caused reduction in expression of GATA3 mRNA and protein in Th2 cells [37]. Thus, stimulation through TCR and IL-4 induced a characteristic sequence of STAT6 activation, dissociation of PcG proteins from chromatin, increase of GATA3 expression, recruitment of TrxG proteins to chromatin and maintenance of GATA3 in the absence of STAT6 (Figure 2) [37]. These studies indicate that the cooperation of histone methyltransferases and lineage-specifying transcription factors is

essential for converting the stimulation of TCR and cytokines signals to the T-cell-intrinsic transcriptional programs.

Whether similar mechanisms are engaged in establishing the transcriptional program specific to the development and maintenance of Th1 cells and Th17 cells has not been reported to our knowledge (Figure 2). Data from recent studies indicate that T-bet can control appropriate cellular differentiation and specification through reversing and establishing new epigenetic states in T cells [115,116]. T-bet recruited the histone methyltransferase SET7/9 to catalyze permissive H3K4me2 at the *IFNG* and *CXCR3* promoters in Th1 cells [115,116]. Interestingly, T-bet also recruited JMJD3 to mediate demethylation of H3K27me3 across the *IFNG* locus (Figure 2) [116]. STAT4 and IL-12 have been shown to be required for Th1-cell differentiation under physiological conditions, as their deficiency dramatically reduces this process [117]. STAT4 bound to the *IFNG* promoter and many other elements (e.g., conserved noncode sequences; CNS-22) and induced permissive DNA modifications [14]. Thus, it appears that epigenetic regulation of Th1-cell differentiation involves a complex and functional cooperation of histone methyltransferase, demethylase and transcription factors [14,115,118], which remains not well understood.

Repressing inappropriate expression of lineage-specific genes

Repressing inappropriate activation of lineage-specific genes has been thought to be crucial for preventing the occurrence of aberrant immune responses [6,13,35,39,60]. SUV39H1-deficient Th2 cells were defective at silencing the *IFNG* and *TBX21* genes and impaired lineage commitment and increased instability in developed Th2 cells when they were re-exposed to Th1-skewing stimuli [39]. This supports the idea that histone methyltransferases may play important roles in repressing inappropriate expression of lineage-specific genes. Some studies suggested that a cooperation of histone methyltransferases and GATA3 may also contribute to the repression of inappropriate expression of lineage-specific genes. For instance, upon Th2-cell differentiation, IFN- γ production is inhibited [11,13,14,119]. This was recently found to be associated with the binding of GATA3 and STAT6 to the *IFNG* promoter and recruitment of PRC1, Ezh2 and H3K27me3 to the *IFNG* locus [26]. This suggests that Ezh2 might be recruited to repress *IFNG* transcription, although loss-of-function experiments have yet to be performed to formally test this possibility. An alternative pathway of repressing IFN- γ production could be achieved through inhibiting the transcription factor STAT4, which is known to be critical for Th1-cell differentiation [11,13,14,119]. Many studies have shown that GATA3 effectively repressed *STAT4* transcription in activated T cells, in particular at the early stage of Th1 differentiation [37,120]. Ectopic expression of GATA3 reduced the expression and activity of STAT4 in Th1 cells [37,121,122], whereas loss of *GATA3* led to increased production of IFN- γ in CD4⁺ T cells under Th2 cell culture conditions [119,123,124]. Additional studies also showed that GATA3 inhibited T-bet function [123,125]. It will be intriguing to investigate if GATA3-mediated repression of STAT4 might require the presence of functional histone methyltransferases and methylation of histones.

T-bet also silences the expression of GATA3 in Th1 cells [14]. Recent studies suggested that T-bet was required for silencing *IL4* in Th1 cells mainly by cooperatively binding with RUNX3 to the *IL4* silencer [14]. Whether histone methyltransferases and how they may be required for T-bet to silence *GATA3* and Th2 cytokine genes has not been reported. Nevertheless, these observations are consistent with an emerging theme that histone methyltransferases may play an important role in setting up chromatin as a signal integration and storage platform [18].

Histone methyltransferase & T-cell immunity

Airway inflammation

A better understanding of how histone methyltransferases and histone methylation regulate T-cell immunity has significant implications for the development of novel strategies to prevent and treat T-cell-mediated inflammatory disorders and to augment cancer immunotherapy [14,126]. Several studies using genetic approaches suggested that inhibition of MLL, SUV39H1 or Bmi-1 led to impaired memory Th2 responses and the subsequent reduction of airway inflammation [25,29,39]. MLL is important in controlling the ability of memory Th2 cells to produce Th2 cytokines. Challenge of MLL^{+/-} mice with ovalbumin (OVA) inhalation induced significantly less severe airway inflammation than MLL-sufficient mice [25], including decreased levels of IgE and IgG1 (Th2-dependent isotypes) antibodies, reduced number of total-infiltrating cells in the airway, and significantly reduced the number of eosinophils in the lung [25]. The histological examination showed that the extent of inflammatory leukocyte infiltration in the peribronchiolar region was much less in the MLL^{+/-} memory Th2 mice compared with mice with MLL-sufficient memory Th2 cells [25]. Similarly, reduced airway inflammation was observed in MLL^{+/-} mice with an OVA-specific CD4⁺ T-cell TCR-transgenic background [25].

Recent studies investigated the effect of SUV39H1 deficiency on Th2-type responses in a model of OVA-induced allergic asthma in mice [39]. Immunological sensitization of WT mice to OVA led to the generation of an antigen-specific Th2 response characterized by OVA-specific production of IL-4 and low levels of IFN- γ in the spleen [39]. In SUV39H1-deficient mice, however, in addition to the generation of IL-4-producing T cells, there was an abundance of allergen-specific IFN- γ ⁺ T cells and IFN- γ ⁺IL-4⁺ dual-producing T cells, indicating skewed Th-cell development [39]. As a result, the airway inflammation was significantly reduced in SUV39H1 mice [39]. In a separate study, it was shown that deletion of Bmi-1 led to impaired memory Th2-cell responses and their associated airway inflammation in mice was challenged by inhalation of OVA [29]. Since pharmacological compounds have been developed to target MLL for the treatment of leukemia [127], it will be interesting to test if pharmacologic inhibition of MLL may modulate allergic inflammation such as asthma.

Infection

Histone methyltransferases may play important roles in regulating immunity in mice infected with the intestinal helminth *Trichuris muris* [35]. G9a was found to be important for promoting Th2-cell differentiation and repressing the development of Th1 and Th17 cells [35]. In mice infected with *T. muris*, Th2-cell responses were required to mediate resistance to infection [128]. By contrast, Th1 cells were associated with chronic infection [128]. G9a-deficient mice had impaired capability to produce Th2-lineage cytokines (IL-4, IL-5 and IL-13) and failed to protect against *T. muris* infection [35].

Graft-versus-host disease

Histone methylation may also play critical roles in regulating allogeneic inflammatory responses. Graft-versus-host disease (GVHD) is a life-threatening complication after allogeneic bone marrow transplantation [5,7,129]. GVHD is caused by infused donor T cells that recognize and react to the histocompatibility differences between the donor and the host [5,7,129]. A recent study suggested that pharmacologic inhibition of histone methylation (e.g., H3K27me3, H3K4me3 and H4K20me3) caused selective apoptosis in allo-antigen-activated T cells and arrest of ongoing GVHD [40]. This pharmacologic inhibition of histone methylation was associated with depletion of the histone methyltransferase Ezh2 and activation of the proapoptotic gene *BIM* repressed by Ezh2 in antigenic-activated T cells

[40]. These findings indicate that pharmacological modulation of histone methyltransferases and histone methylation pathways may lead to new strategies to treat inflammatory disorders, such as autoimmune diseases, chronic infection and rejection of allo-geneic grafts after organ transplantation in a broad context. Recently, several studies reported the discovery of new pharmacologic inhibitors that can selectively and specifically inhibit Ezh2 enzymatic activity [130–132]. This will further facilitate testing our hypothesis.

Conclusion & future perspective

Many studies have established the correlation of histone methylation signatures with the development and maintenance of effector and memory T cells. Future studies should investigate the function of histone methyltransferases and the resulting regulation of histone methylation in T-cell immune responses. Experiments from these functional studies will be essential for addressing how histone methyltransferases regulate lineage commitment, plasticity and stability of both CD4⁺ and CD8⁺ T cells during immune responses; for example, a unique feature of antigen-activated CD8⁺ T cells is the production of heterogeneous cell populations, including short-lived effector cells and long-lived memory precursor cells [8]. Recent studies demonstrate that cooperative maintenance of antigen-experienced CD8⁺ T cells with different properties is crucial for the development of adaptive immunity against persistently expressed antigens, such as reactions to chronic infection, tumor cells and alloantigens [9,60,133–138]. Epigenetic effects are believed to be important for orchestrating transcriptional programs to induce the generation of different subsets of antigen-activated CD8⁺ T cells in response to different environmental stimuli [8,14]. It will be intriguing to determine the function of histone methyltransferases in antigen-driven CD8⁺ T cells.

A given histone methyltransferase may affect multiple transcriptional programs [18, 36,37,72,76,108]. Moreover, some histone methyltransferases may function at levels beyond catalyzing histone methylation. For example, Ezh2 may participate in mediating cell signaling through methylation of other signaling proteins [72,89,139], interacting with NF- κ B to regulate gene expression in the absence of enzyme activity [140], and recruiting DNMT3a and DNMT3b (which mediate DNA methylation) to repress gene expression [110]. G9a can regulate production of Th2 cytokines independent of its histone methyltransferase enzyme activity [35]. Thus, novel approaches are needed to dissect the impact of different molecular pathways in mediating histone methyltransferase effects on T-cell responses.

Epigenetic effects have been thought to play crucial roles in converting environmental signals to cell-intrinsic programs that regulate expression of genes in T cells. These genes are associated with multiple cellular processes [14,31,33,37,72]. Understanding how histone methyltransferases and their catalyzed histone methylation pathways convert environmental stimuli to T-cell-intrinsic programs is not only inherently interesting, but will also lead to new strategies for modulating T-cell immunity.

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Executive summary

Histone methylation landscape in CD4⁺ T cells

- Histone methylation marks effector cytokine genes in CD4⁺ T cells.
- Histone methylation marks transcription factor genes in CD4⁺ T cells.

Histone methylation signatures in CD8⁺ T cells

- Histone methylation marks genes encoding effector molecules in CD8⁺ T cells.
- Histone methylation marks transcription factor genes in CD8⁺ T cells.

Function of histone methyltransferase in effector cells

- G9a plays differential roles in Th1 and Th2 cells.
- SUV39H1 sustains the stability of Th2 cells.
- MLL regulates the development and maintenance of memory Th2 cells.
- Ezh2 is associated with differentiation of distinct subsets of effector T cells.

Histone methyltransferase maintaining of effector function in memory cells

- Active chromatin marks are associated with the effector gene loci in memory cells.
- Histone methyltransferases regulate the properties of memory cells.
- Histone methyltransferases orchestrate gene programs critical for the survival of memory cells.

Converting extrinsic signals to T-cell-intrinsic programs

- Histone methyltransferases and transcription factors cooperatively regulate lineage differentiation.
- Histone methyltransferases prevent inappropriate expression of lineage-specific genes.

Histone methyltransferases & T-cell immunity

- MLL and SUV39H1 regulate T-cell-mediated airway inflammation.
- G9a promotes Th2 cell-mediated immunity against *Trichuris muris*.
- Histone methylation is critical for alloimmunity.

Figure 1. Histone methylation signatures for lineage-specific cytokines in effector CD4⁺ T cells
(A) Naive CD4⁺ T cells are characterized by histone methylation signatures consistent with the quiescent state. In these cells, moderate levels of repressive trimethylated (me3) H3K27 were present at the *IFNG* locus and the *IL4* locus. Upon Th1-cell differentiation, the regulatory region of *IFNG* showed a reduction of H3K27me3, but an increased expression of both the active mark H3K4me3 and repressive mark dimethylated H3K9. In Th1 cells, H3K27me3 extensively marked the regulatory regions of the *IL4* and *IL17A* gene loci. Th2 cells showed a reduction of H3K27me3 and a strong induction of H3K4me3 in the *IL4* locus. H3K27me3 was evident in the *IFNG* and *IL17A* loci. Furthermore, dimethylated H3K9 was induced in the *IFNG* locus early during Th2 differentiation. Th17 cells were characterized by the presence of H3K4me3 at the *IL17A* promoter and a high level of H3K27me3 at the *IFNG* and *IL4* loci. **(B)** Naive CD8⁺ T cells had abundant H3K27me3 but low levels of H3K4me3 at the promoter of the *IFNG* locus, and high-expression of H3K27me3 in *GZMB* and *PRF1* loci. By contrast, they showed low amounts of H3K27me3 and high levels of H3K4me3 at the proximal promoter of *TNF-α*. Upon differentiation into CTLs, the *IFNG* promoter showed reduction of H3K27me3 but markedly enriched H3K4me3. The *GZMB* locus, particularly transcribed regions, was strongly marked with H3K4me3 and H3K9me3. CTLs showed no significant alteration of repressive mark H3K27me3 at the promoter of *PRF1*.
 CTL: Cytotoxic T lymphocyte.

Figure 2. Histone methyltransferases and transcription factors cooperatively regulate the differentiation of distinct lineages of effector CD4 T cells

(A) In Th1 cells, an abundance of trimethylated (me₃) H3K4 occurs at the *TBX21* locus. However, whether and how histone methyltransferases regulate *TBX21* transcription in Th1 cells remains largely unknown. In these Th1 cells, T-bet directly binds to the *IFNG* promoter, resulting in recruitment of JMJD3 to remove H3K27me₃. Binding of T-bet to chromatin also forms a complex with SET7/9, which catalyzes the permissive histone mark dimethylated H3K4. Whether STAT4 can directly recruit certain histone methyltransferase(s) to the regulatory region of *IFNG* to modify gene expression remains unclear. (B) In Th2 cells, activation of STAT6 leads to dissociation of Ezh2 and other PcG proteins from the *GATA3* locus. This is accompanied by the recruitment of the Menin–TrxG complex to the whole *GATA3* gene region and subsequent activation of *GATA3* transcription. Once developed, Th2 cells acquire the ability to prevent the reassociation of Ezh2–PcG complex with the *GATA3* locus via an unknown mechanism. In terms of the *IL13/IL4* locus, active histone methylation such as H3K4me₃ is acquired during Th2 differentiation, which is accompanied with recruitment of MLL. G9a, which can catalyze repressive dimethylated H3K9, is also recruited to the *IL13/IL4* locus. (C) In Th17 cells,

both the *RORC* and *IL17A* gene loci are marked by permissive mark H3K4me3. However, which histone methyltransferase(s) and how they are recruited to these regions have yet to be determined. me2: Dimethylated; me3: Trimethylated.

Table 1

Histone methyltransferases and their methylated lysines at histones.

Histone and residue	Histone methyltransferase	
	<i>me2</i>	<i>me3</i>
H3K4	SETD1A, SETD1B, ASH1L, MLL, MLL2, MLL3, MLL4, SMYD3, SET7/9	SETD1A, SETD1B, ASH1L, MLL, MLL2, MLL3, MLL4, SMYD3, PRMD9, SET7/9
H3K27	Ezh2, Ezh1	Ezh2, Ezh1
H3K9	SUV39H1, SUV39H2, SETDB1, PRDM2, G9a, EHMT1	SUV39H1, SUV39H2, SETDB1, PRDM2
H3K36	SETD2, NSD1, NSD2, NSD3, SMYD2	SETD2
H3K79	DOT1L	DOT1L
H4K20	SUV420H1, SUV420H2	SUV420H1, SUV420H2

me2: Dimethylated; *me3*: Trimethylated.

Adapted with permission from [68].