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REVIEW

Regulation of CD4 T-cell differentiation and inflammation by repressive histone methylation

Frann Antignano and Colby Zaph[1]

Repressive epigenetic modifications such as dimethylation and trimethylation histone H3 at lysine 9 (H3K9me2 and H3K9me3) and H3K27me3 have been shown to be critical for embryonic stem (ES) cell differentiation by silencing cell lineage-promiscuous genes. CD4$^+$ T helper (T_H) cell differentiation is a powerful model to study the molecular mechanisms associated with cellular lineage choice in adult cells. Naïve T_H cells have the capacity to differentiate into one of the several phenotypically and functionally distinct and stable lineages. Although some repressive epigenetic mechanisms have a critical role in T_H cell differentiation in a similar manner to that in ES cells, it is clear that there are disparate functions for certain modifications between ES cells and T_H cells. Here we review the role of repressive histone modifications in the differentiation and function of T_H cells in health and disease.
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Gene silencing is a complex and coordinated process that is critical for generating and maintaining cellular lineage identity. Several distinct molecular mechanisms have been identified that repress gene expression during lineage commitment. These epigenetic mechanisms include microRNA expression, DNA methylation and histone modifications that have been extensively analyzed during stem cell differentiation. In the immune system, CD4$^+$ T helper (T_H) cells also undergo differentiation from a 'naïve' multipotent progenitor cell into one of the several functionally distinct lineages. Although it is clear that similar epigenetic mechanisms operate in T_H cells,[1–3] there is much less known about how repressive epigenetic mechanisms modulate the phenotype and function of T_H cells.

T_H CELLS: A MODEL FOR CELLULAR LINEAGE CHOICE

One of the hallmarks of the immune system is the ability of immune cells to modulate their functional capacity in response to external stimuli. Perhaps the best characterized are T_H cells, lymphoid cells that have the capacity to differentiate into one of the several lineages, including T_H1, T_H2, T_H17 and inducible regulatory T (iT_{reg}) cells, depending on the anatomical location, cytokine milieu and other intrinsic and extrinsic factors.[4,5] After exiting the thymus, 'naïve' T_H cells encounter antigen-bearing major histocompatibility complex II-expressing immune cells such as dendritic cells that promote the proliferation and differentiation of T_H cells into distinct functional subsets. For T_H1 cell development, signal transducer and activator of transcription factor 1 (Stat1) activation by interferon (IFN)-γ results in the expression of Tbx21 (T-bet), the master regulator of T_H1 lineage choice.[6] Tbx21 induces the transcription factor Runx3 and interleukin (IL)-12 receptor β2 leading to Stat4 activation, which in combination with Tbx21 and Runx3 induces IFN-γ expression and T_H1 lineage commitment.[7–9] At the same time, Tbx21 and Runx3 repress *Il4* expression and T_H2 development. For T_H2 cells, IL-4 signaling activates Stat6, which leads to Gata3 expression.[10] Gata3 activates c-maf,[10] and together these transcription factors activate expression of the *Il13–Il4* locus. In addition, Stat6 and Gata3 repress *Ifng* expression, further enforcing T_H2 lineage commitment.[10] T_H17 cell development is controlled by the nuclear hormone receptors retinoic acid-related orphan receptor α (RORα) and RORγt, which are induced by IL-6 and transforming growth factor β (TGFβ).[11,12] Cytokines, including IL-21 and IL-23, activate Stat3 and act to stabilize the T_H17 lineage, while the aryl hydrocarbon receptor is also critical for the induction of the T_H17 lineage.[13–19] TGFβ induces expression of the transcription factor Foxp3 that controls T_{reg} cell development. Thus T_H cell lineage commitment is a tightly regulated process controlled by lineage-specific sets of inducible transcription factors. Although the molecular mechanisms controlling the lineage choice of T_H cells—such as the expression of lineage-specific transcription factors—are better understood, recent studies have highlighted the critical role of epigenetic mechanisms in T_H cell differentiation.

T_H CELLS: CENTRAL MEDIATORS OF INFLAMMATORY DISEASE

Dysregulated T_H cell responses are a characteristic of several inflammatory diseases, including inflammatory bowel disease, arthritis, diabetes, asthma and allergies. T_H1 cells have been associated with several inflammatory conditions, including arthritis, inflammatory bowel disease and diabetes.[20,21] Overproduction of type 2 cytokines by T_H2 cells can lead to pathological conditions, including asthma and allergies.[22–25] Dysregulated T_H17 and iT_{reg} cell responses have also been found in a wide variety of inflammatory conditions, including

The Biomedical Research Centre, University of British Columbia, Vancouver, BC, Canada
[1]Current address: Department of Biochemistry and Molecular Biology, Monash University, Wellington Road, Clayton, VIC 3800, Australia.
Correspondence: Dr C Zaph, The Biomedical Research Centre, University of British Columbia, 2222 Health Sciences Mall, Vancouver, BC, Canada V6T1Z3.
E-mail: colby.zaph@monash.edu
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multiple sclerosis, rheumatoid arthritis and inflammatory bowel disease.[26] Thus a better understanding of the molecular mechanisms that control T_H cell lineage choice may provide novel therapeutic targets to treat this wide range of inflammatory diseases.

CHROMATIN STRUCTURE AND MODIFICATIONS

The fundamental unit of chromatin is the nucleosome, comprised of 147 base pairs of DNA wrapped around two of each of the core histones H2A, H2B, H3 and H4.[27,28] The dynamics involved in DNA-associated processes such as transcription are regulated by both modifications of the DNA itself through cytosine methylation as well as a wide variety of posttranslational modifications deposited on histones. DNA methylation patterns at cytokine gene loci are associated with inhibitory chromatin.[2,10,29–40] For example, in T_H1 cells, the *Il4* locus is DNA methylated while the *Ifng* gene is methylated in T_H2 cells. In contrast, histone modifications are biochemically very diverse and can decorate serine, lysine and arginine residues in the N-terminal tails of histones H3 and H4 and in the core regions of histone H2A and H2B, include phosphorylation, acetylation, sumoylation, ubiquitination and methylation and can be associated with active or silenced genes.[41]

Among all the chromatin modifications, the site-specific methylation of histone lysines has emerged as a fundamental regulatory mechanism.[42] Lysine residues can be monomethylated, dimethylated or trimethylated resulting in a number of possible combinations that equips the cell for the establishment of numerous specialized chromatin states. In general, actively transcribed genes are demarcated with promoter-associated peaks of tri-methylated histone H3-Lysine 4 (H3K4me3) whereas silent genes are embedded in large domains of H3K9me2, H3K9me3 or H3K27me3 marks.[43] In this review, we discuss the specific role of repressive histone modifications mediated by methylation of H3K9 and H3K27 in T_H cell differentiation and function.

REPRESSIVE HISTONE LYSINE METHYLATIONS

The catalytic domain of almost all histone lysine methyltransferases is the SET domain, a highly conserved motif originally found in the modifier of position effect variegation (SU(VAR)3-9), the polycomb-group protein enhancer of zeste (E(z)) and the trithorax-group protein trithorax (TRX).[44] There are approximately 50 members of the SET-domain family, all with distinct abilities to mono-, di- or tri-methylate specific lysines of histones and, in some cases, non-histone proteins. Studies have shown that polymethylation of H3K9 and H3K27 are linked to gene repression, and these marks are often found to accumulate on lineage-promiscuous genes during development.[43,45] Here, we focus on three distinct methyltransferases that control repressive histone modifications—G9a, Suv39h1 and Ezh2 (Figure 1)

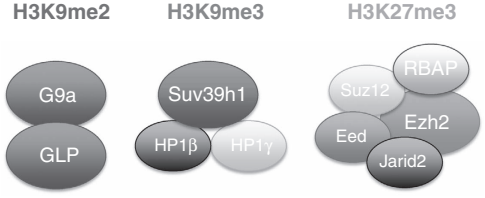

Figure 1 The enzyme complexes involved in repressive histone methylation in T_H cells. A full colour version of this figure is available online at the *Immunology and Cell Biology* website.

—and define their roles in T_H cell differentiation and function in health and disease.

G9A

G9a (Ehmt2, Kmt1c) is a histone methyltransferase with monomethylation and dimethylation activities towards H3K9.[46] G9a forms a heterodimer with the related protein G9a-like protein (GLP, Ehmt1, Kmt1b). This heterodimerization is critical as either G9a or GLP deficiency results in a significant reduction in H3K9me2 levels.[47] Although there is a significant overlap in dysregulated gene expression when comparing deficiency in G9a or GLP, there are some differences,[48] suggesting that there are some heterodimer-independent roles. However, G9a will be the focus of this review. A number of studies have demonstrated that G9a is crucially involved in cellular differentiation and development. For example, G9a-deficient mice do not develop beyond day 8.5 of gestation[46] and $G9a^{-/-}$ embryonic stem (ES) cells are characterized by widespread alterations in DNA methylation patterns and promiscuous expression of numerous genes.[49,50] In addition, the sustained silencing of pluripotency-associated genes in $G9a^{-/-}$ ES cells is impaired and results in the reversal from a differentiated state into a pluripotent state in a significant fraction of cells.[51,52] Chromatin immunoprecipitation sequencing studies in both ES cells and hematopoietic stem cells have shown that overall genome-wide levels of G9a-dependent H3K9me2 are low in undifferentiated stem cells and is acquired as cells differentiate.[53,54] These data were interpreted to suggest that H3K9me2 is acquired at lineage-promiscuous loci to promote lineage integrity and block de-differentiation to a pluripotent progenitor state.[52] Indeed, inhibition of the enzymatic activity of G9a with a chemical probe can replace Oct3/4 overexpression and increases the efficiency of reprogramming over eightfold.[55,56]

However, it remains unknown how G9a is recruited to chromatin to carry out these roles. Although G9a does not have a DNA-binding domain, it does have the ability to bind to dimethylated lysine residues, including H3K9me2, through its ankyrin repeat domain.[57] In addition, several G9a-interacting proteins with DNA-binding activity have been identified, including Gfi1 and Blimp1.[58,59] Strikingly, these G9a-binding partners are directly involved in T_H cell differentiation and activation. Gfi1 is critical for differentiation of both T_H cells and innate lymphoid cells.[60–63] $Gfi1^{-/-}$ T_H cells fail to develop into T_H2 cells[60] owing to decreased stability of Gata3 protein.[61] Blimp1 has an important role in T_H cell function by controlling the expression of several cytokines, including IL-2, IL-10 and IL-17A.[59,64–68] Taken together, a general theme has emerged where G9a has a role in the epigenetic silencing of cell-type inappropriate genes through the interaction with a wide variety of DNA-binding proteins.

SUV39H1

Trimethylation of H3K9 in mice is carried out by suppressor of variegation 3-9 homologue 1 and 2 (Suv39h1/2, Kmt1a/b) histone methyltransferases, which are encoded by two loci, *Suv39h1* and *Suv39h2*.[69] Suv39h1/2 was the first mammalian methyltransferase described[70] and is homologous to yeast Clr4 and Drosophila Su (Var)3-9.[71,72] During embryogenesis, *Suv39h1* and *Suv39h2* show overlapping expression profiles, while in adult mice *Suv39h1* is the primary methyltransferase expressed in non-testes.[69] Suv39h1-dependent trimethylation of H3K9 is associated with heterochromatin found at centromeric and telomeric regions as well as gene-poor areas (gene deserts) and repetitive regions.[43] Functionally, the addition of H3K9me3 generates a binding site for the methyl-lysine-binding

protein heterochromatin protein 1 (HP1).[73,74] HP1 promotes transcriptional repression at and near heterochromatin (position-effect variegation) as well as at euchromatin.[75] Mechanistically, it has been suggested that HP1 recruits histone deacetylases to prevent epigenetic activation of specific loci.[76] Mice deficient for either *Suv39h1* or *Suv39h2* display normal viability and fertility with no obvious phenotypes.[77]

EZH2

Enhancer of zeste homologue 2 (Ezh2, Kmt6) is the primary H3K27 trimethyltransferase.[78] In association with a large complex of proteins called the Polycomb Repressive Complex 2, Ezh2—but not its close homologue Ezh1—is required for the trimethylation of H3K27.[79] Ezh2-dependent H3K27me3 has been implicated in several forms of cancer, including prostate cancer,[80] breast cancer,[81] T-acute lymphoblastic leukemia[82] and diffuse large B cell lymphoma.[83] The primary role for Ezh2 and H3K27me3 is in repression of gene expression in euchromatin.[84] Ezh2 has a critical role in B- and T-cell development, as Ezh2-deficient mice have significantly reduced frequencies of B and T cells in peripheral lymphoid tissues.[85,86] As discussed below, Ezh2-mediated H3K27me3 has a central role in T_H cell differentiation and function.

GENE REPRESSION DURING T_H CELL DIFFERENTIATION

Gene silencing is an essential molecular mechanism to promote and maintain cellular lineage integrity. The majority of studies in T_H cells have focused on the role of repressive DNA methylation marks mediated by Dnmt1 and Dnmt3a/b.[2,10,29–40] From these studies, it is clear that silencing of inappropriate gene expression by DNA methylation is critical in T_H cell differentiation. However, the role of repressive histone modifications remains less clear.

The genome-wide expression of Ezh2-dependent H3K27me3 marks during T_H cell differentiation has been described.[87] These studies demonstrated that lineage-promiscuous genes are decorated with H3K27me3 (for example, *Il4* in T_H1 cells and *Ifng* in T_H2 cells). However, a striking finding in this report was that H3K27me3 was a relatively rare mark that was not always present on non-expressed genes. For example, in T_H2 cells, out of a selected set of 40 lineage-promiscuous genes, <10 genes showed any measurable levels of H3K27me3. In T_H17 cells, only 5 out of the 44 non-expressed genes were positive for H3K27me3. Thus, it is clear from these studies that additional repressive modifications were likely to co-regulate gene silencing at these loci in T_H cells (Figure 2).

DYNAMIC EXPRESSION OF REPRESSIVE HISTONE MODIFICATIONS

G9a-dependent H3K9me2

Although studies have begun to elucidate the role of epigenetic mechanisms in T_H cell development,[38,87–89] the function of G9a-dependent H3K9me2 marks is less clear. A descriptive genome-wide analysis of H3K9me2 marks in resting human lymphocytes using chromatin immunoprecipitation-on-chip methods demonstrated that this epigenetic mark is enriched on genes that are associated with several specific pathways, including T-cell receptor signaling, IL-4 signaling and Gata3 transcription.[90] Based on studies in ES cells and hematopoietic stem cells, it was likely that G9a-dependent H3K9me2 would have a critical role in maintenance of lineage integrity in T_H cells. In contrast to expectations, it is clear that G9a is not necessary for lineage-promiscuous gene silencing in T_H1, T_H2, T_H17 and iT_{reg} cells.[91,92] Strikingly, very low levels of H3K9me2 were found at all the genes examined in activated T_H cell lineages. However, H3K9me2 is enriched at many diverse loci in naïve, unstimulated T_H cells. Following activation, H3K9me2 was rapidly lost at all loci examined, including lineage-promiscuous loci, without leading to promiscuous gene expression. These results suggest a radical reimagining of the role of G9a-dependent H3K9me2 in T_H cell differentiation, where loss of repressive chromatin modifications is required for normal T_H cell differentiation and function.

Suv39h1-dependent H3K9me3

Despite being the first methyltransferase identified, there are very few studies on the role of Suv39h1 in immune cells, and until recently, the functional relevance of this modification in T_H cell differentiation was unknown. Similar to H3K9me2, H3K9me3 is found at high levels in naïve T_H cells and increases at lineage-promiscuous loci (*Il4* and *Gata3* loci upon differentiation into T_H1 cells and *Ifng* locus in T_H2 cells).[93] The addition of H3K9me3 by Suv39h1 provides a binding site for heterochromatin protein 1α (HP1α) to promote gene silencing.

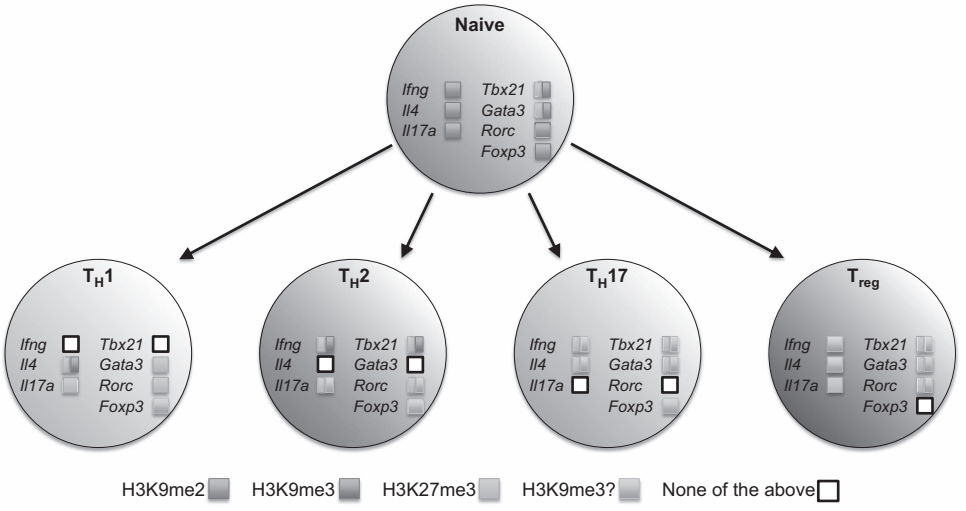

Figure 2 Dynamic regulation of repressive histone methylation in T_H cells. A full colour version of this figure is available online at the *Immunology and Cell Biology* website.

Therefore, H3K9me3 and HP1α accumulate at the promoters of the genes characteristic of the opposite lineage—a traditional effect of transcriptional repressors. This pattern of repression of lineage-promiscuous genes is similar to the role of G9a-dependent H3K9me2 in ES cells (but, as described above, not in T_H cells). Thus Suv39h1-dependent H3K9me3 appears to be a critical component of lineage integrity in T_H1 and T_H2 cells by silencing lineage-promiscuous gene expression.[93]

Ezh2-dependent H3K27me3

Although Ezh2 is one enzyme responsible for the trimethylation of H3K27, initial studies of its role in T cells found no difference in the global levels of H3K27me3 in the absence of Ezh2, suggesting that Ezh1 may be compensating for its loss.[86] Despite this, Ezh2 did have a role in promoting T-cell survival through the methylation of Vav, which was required for actin polymerization during T-cell receptor signaling. An early study looking at the role of Ezh2-mediated H3K27me3 found this modification to be associated with *Il4* and *Il13* gene silencing in T_H1 cells.[94] Again, similar to the results with H3K9me2 and H3K9me3, high levels of H3K27me3 were found at the *Il4-Il13* locus in naïve T_H cells that were progressively lost during T_H2 cell differentiation. Meanwhile, H3K27me3 was retained at the *Il4-Il13* locus in T_H1 cells. Similar data were obtained when H3K27me3 levels were globally mapped in different T-cell lineages.[87] Using chromatin immunoprecipitation sequencing, this study found selective loss of H3K27me3 on lineage-specific cytokine and transcription factor genes and retention or accumulation on lineage-promiscuous genes. In a recent study, however, a specific loss of H3K27me3 at lineage-specific sites was not observed, but rather H3K27me3 was reduced from naïve levels at both lineage-specific and -non-specific loci following differentiation into T_H1 and T_H2 cells.[95] In addition, Ezh2 has been found to bind to *Tbx21* and *Gata3* gene loci.[96] Specifically, Ezh2 binds to the *Gata3* locus in naïve and T_H1 cells and binding is significantly reduced in T_H2 cells. Likewise, Ezh2 binds to the *Tbx21* locus in naïve and T_H2 cells but is absent in T_H1 cells. It was also observed that H3K27me3 was lost at T_H2 cell cytokine gene loci in T_H2 cells and at the *Ifng* locus in T_H1 cells.[96] As expected, multiple groups have shown that H3K27me3 is dramatically reduced at T_H1 and T_H2 cell-associated genes in the absence of Ezh2 in T cells.[95–97] Thus, although there are discordant results, it is clear that H3K27me3 is dynamically regulated during T_H cell differentiation and likely has a critical role in T_H cell lineage development and integrity.

Taken together, these data suggest that naïve T_H cells inherently harbor a high level of repressive histone modifications that are removed following activation and differentiation. The exact mechanisms that control the dynamic regulation of H3K9me2, H3K9me3 and H3K27me3 during T_H cell differentiation remain to be elucidated. For example, the loss of repressive modifications during T_H cell activation implies a critical role for the expression and activation of specific lysine demethylases[98] that would have an essential role in promoting functional T_H cell responses. Nevertheless, it is apparent that a better understanding of the regulatory functions of these modifications is important for defining the molecular mechanisms that control T_H cell function in health and disease.

REPRESSIVE HISTONE MODIFICATIONS IN T_H CELL DIFFERENTIATION

G9a-dependent H3K9me2 limits T_H17 and T_{reg} cell development

Following polyclonal stimulation of purified G9a-deficient $CD4^+$ T cells, significantly increased frequencies of IL-$17A^+$ (under T_H17 cell conditions) and $Foxp3^+$ (under iT_{reg} cell conditions) cells were observed. G9a deletion has no effect on the development, phenotype or function of thymically derived 'natural' T_{reg} cells.[92] The effects of G9a on T_H17 and T_{reg} cell differentiation are dependent upon the methyltransferase activity of G9a. Activation of wild-type T_H cells in the presence of a specific G9a inhibitor resulted in heightened frequencies of both T_H17 and T_{reg} cells. These results suggested that the effects observed were due to dysregulated histone methylation.

TGFβ is required for differentiation of both T_H17 and iT_{reg} cells.[26] Under iT_{reg} cell-promoting conditions, a 20-fold increase in sensitivity to TGFβ in G9a-deficient T_H cells was observed. The heightened responsiveness was not due to differences in the expression of TGFβ receptors or proximal signaling pathways between G9a-sufficient and -deficient T_H cells. Thus, G9a controls T_H17 and T_{reg} cell differentiation through the epigenetic regulation of TGFβ responsiveness.

G9a regulates chromatin accessibility

Using an unbiased genome-wide analysis of chromatin structure called formaldehyde-assisted isolation of regulatory elements sequencing,[99] chromatin accessibility in the presence or absence of G9a was analyzed. It was found that the chromatin surrounding the *Foxp3* promoter and conserved non-coding sequence-1 (TGFβ-responsive element) in naïve G9a-deficient T_H cells showed increased accessibility, as measured by increased sequence reads at these sites. Thus G9a is critical for controlling TGFβ sensitivity, potentially through regulating chromatin accessibility.

G9a-dependent H3K9me2 has been shown to be associated with chromatin located in close proximity to the nuclear lamina.[100–102] This localization is a physical form of gene silencing, as genes physically located at the periphery of the nucleus tend to be expressed at significantly lower levels due to decreased accessibility of transcriptional coactivators, chromatin modifiers and transcription factors.[103] These lamina-associated domains (LADs) are large (0.1–10 Mb) regions of chromosomes demarcated by the genomic insulator CCCTC- binding factor that display low levels of gene expression.[100] Consistent with the results above, LADs are marked with high levels of the histone modification H3K9me2, mediated by G9a.[101,102] Thus it is possible that a lack of G9a and H3K9me2 leads to increased chromatin accessibility through a decrease of LADs. Future studies using genetic marking assays (DamID)[100] will identify the specific genetic loci that make up LADs in T_H cells.

Suv39h1 is required for T_H2 cell lineage integrity

Using the genetically deficient mice, it was shown that in the absence of Suv39h1 the differentiation of T_H cells into T_H1 and T_H2 cells *in vitro* was comparable to wild-type controls.[93] This suggests that Suv39h1-mediated gene repression is not required for promoting the expression of lineage-specific genes and differentiation into either T_H1 or T_H2 cells. Normally, T_H1 and T_H2 cells are relatively stable lineages that cannot easily be reprogammed into the opposite lineage. However, unlike wild-type cells, Suv39h1-deficient T_H2 cells were able to express IFN-γ and T-bet when placed in T_H1-promoting conditions. This was in contrast to Suv39h1-deficient T_H1 cells, which were not capable of being reprogrammed when placed in T_H2-promoting conditions. Together, these results suggest a specific role for Suv39h1 in the maintenance of silenced T_H1 signature genes in T_H2 cells.

Ezh2 alters T_H1 and T_H2 cell differentiation and enhanced plasticity

Ezh2 is not required for the development of CD4 or CD8 T cells in the spleen or thymus; however, the function of T_H cells in the absence of Ezh2 was altered.[96] In the absence of Ezh2 (or its Set domain), cells

overproduce IFN-γ under non-polarizing and T_H1-polarizing conditions and overproduce IL-4, -5 and -13 under non-polarizing and T_H2-polarizing conditions.[95,96] There was no promiscuous cytokine production in T_H1 or T_H2 cells, indicating that polarization is not dysregulated in the absence of Ezh2.[95,96] However, under iT_{reg} cell-promoting conditions, the absence of Ezh2 resulted in dysregulated IFN-γ production and reduced frequency of $Foxp3^+$ cells.[95] In addition, Ezh2-deficient T cells, much like Suv39h1-deficient T cells, show enhanced plasticity.[93,96] Unlike Suv39h1, the loss of Ezh2 resulted in enhanced plasticity of both T_H1 and T_H2 lineages.[96] In contrast to these studies, others have found that Ezh2-deficient T cells are impaired in their ability to produce IFN-γ *in vitro* under T_H1 cell-promoting conditions as well as *in vivo* resulting in fewer T_H1 cells and an inability to cause T-cell-mediated aplastic anemia.[97,104] Despite these dichotomous results, these studies demonstrate that Ezh2-dependent H3K27me3 is a central component of T_H cell lineage development and maintenance. The details and mechanisms associated with these effects remain to be elucidated.

REPRESSIVE HISTONE MODIFICATIONS IN DISEASE

T-cell-intrinsic expression of G9a controls pathogenic T_H cell development

In a mouse model of intestinal inflammation where purified naïve T_H cells are transferred into immunodeficient *Rag1*$^{-/-}$ mice resulting in inflammation, cachexia and death,[105] it was shown that G9a was required for these T_H cells to cause disease.[92] Transferred naïve G9a-deficient T_H cells developed into non-pathogenic T_H17 and iT_{reg} cells *in vivo* at a 5–10-fold higher frequency than wild-type T_H cells. These results confirmed *in vitro* studies (described above), which identified a role for G9a in limiting T_H17 and iT_{reg} cell differentiation by controlling chromatin accessibility and TGFβ responsiveness.[92]

Critical role for G9a in T_H2 cell-dependent immunity to helminth infection

In contrast to T_H17 and iT_{reg} cells, it was found that following polyclonal stimulation under T_H2-promoting conditions G9a-deficient T_H cells failed to maximally produce type 2 cytokines, suggesting that G9a may act as a specific and positive regulator of gene expression in T_H2 cells.[91] Consistent with this hypothesis, G9a is required for the optimal development of T_H2 cells *in vivo* using the *Trichuris muris* infection model.[106] Following infection, mesenteric lymph node cells and gut tissue isolated from mice with a T-cell-specific deletion of G9a exhibited reduced expression of protective T_H cell-derived cytokines (IL-4, IL-5 and IL-13), increased IFN-γ expression and failed to clear parasites from the gastrointestinal tract. In strains of mice that show increased susceptibility to *Trichuris*, inhibition of type 1 cytokines through antibody treatment or genetic deletion results in immunity to infection.[106] However, blockade of IFN-γ with a monoclonal antibody failed to induce resistance to infection in T-cell-specific G9a knockout mice, identifying a critical cell-autonomous role for G9a in promoting expression of the T_H2 cell-associated cytokines IL-4, IL-5 and IL-13. Thus G9a is a critical component of the machinery required for the development of functional T_H2 cell responses *in vivo*.

Importantly, despite a failure of G9a-deficient T_H2 cells to produce cytokines, the expression of key T_H2 cell-associated transcription factors was unaffected, with similar expression levels of *Gata3*, *Stat6*, *Gfi1* and *c-maf* by comparison to wild-type T_H2 cells. These results suggest that G9a has an important role in promoting the assembly of a productive transcriptional complex at the T_H2 cell-associated cytokine loci. Consistent with this, a small number of studies have shown that G9a can promote transcriptional activation at certain loci,[107,108] including the β-globin locus, a model genetic locus that has many similarities to the *Il4–Il13* type 2 cytokine locus.[109] Intriguingly, the activating functions of G9a are independent of its enzymatic activity.[108,110] Consistent with an activating role for G9a in type 2 cytokine expression, treatment of wild-type T_H cells with a chemical inhibitor of G9a enzymatic activity[111,112] had no effect on T_H2 cell differentiation or type 2 cytokine expression. These results suggest that G9a but not its methyltransferase activity is required for optimal T_H2 cell differentiation. Recently, it has been demonstrated that the previously uncharacterized N-terminal domain (amino acids 1–333) of G9a is necessary and sufficient for the transactivating functions of G9a.[113] Taken together, these results suggest a model in T_H2 cells whereby the N-terminal region of G9a is important to bring together a transcriptional complex leading to expression of type 2 cytokine genes. Interaction between G9a, Gfi1 and possibly other factors such as Gata3 would coordinately activate the *Il4–Il13* locus.

Reduced lung inflammation in the absence of Suv39h1

To investigate the functional outcome of plastic T_H2 cells in the absence of Suv39h1, a model of ovalbumin (OVA)-induced T_H2-dependent allergic lung inflammation was used.[114] Suv39h1-deficient mice were protected from this T_H2 model as indicated by reduced eosinophilic infiltration and mucous production in the airways compared with wild-type control mice. In addition, Suv39h1-deficient mice also showed an increase in T_H1 cell responses that are uncharacteristic of this model.[93] Excitingly, the authors also show that inhibiting Suv39h1 with the specific inhibitor chaetocin[115] in wild-type mice recapitulated the phenotype of Suv39h1-deficient mice in response to T_H2-medicated allergic airway inflammation.

Ezh2 controls survival of T_H cells and limits asthma pathology

In vitro results had indicated that Ezh2-deficeint T_H cells displayed increased cell death over time following activation.[95] *In vivo*, this translated into increased cell death of T_H1 cells and a failure to clear listeria infection.[95] This result was consistent with another study where donor Ezh2-deficient T cells underwent apoptotic cell death and failed to elicit graft versus host disease after allogeneic bone marrow transplant in recipient mice.[104] Although the initial proliferation of Ezh2-deficient T cells was normal, their ability to maintain a proliferative state was defective leading to enhanced survival of recipient mice.

Using a model of OVA asthma where *in vitro*-generated OVA-specific T cells are transferred into wild-type mice and challenged with aerosolized OVA, recipients of Ezh2-deficient OVA-specific T_H cells displayed increased eosinophilic infiltration and increased total cells in the airways.[96] In addition, the mice that received Ezh2-deficient T cells also had higher levels of T_H2 cytokines in bronchoalveolar lavage samples and increased lung pathology.

DRUGGING THE EPIGENOME TO TREAT T_H CELL-MEDIATED DISEASE

The realization that the activity of epigenetic readers, writers and erasers can be modulated with drug-like small molecules has led to intense activity over the past several years to identify small-molecule inhibitors that may lead to new 'epigenetic therapies'.[116,117] Although there is much optimism for this approach, our current understanding of epigenetic mechanisms is still in its infancy, and it is not clear which (if any) are the best targets in inflammation or other diseases. In addition, it is clear that, at least for G9a, there are critical functions that are independent of enzymatic activity.

Currently, there are commercially available specific and potent chemical inhibitors of G9a (UNC0638, A-366), Ezh2 (GSK343) and Suv39h1 (chaetocin).[112,115,118,119] However, these probes have not been tested *in vivo* using the models of inflammatory diseases. The development of bioavailable inhibitors with good pharmacokinetics is an important step in the development of epigenetic inhibitors that may be used to modulate T_H cell function during disease.

In addition, the active demethylation of repressive histone marks during T_H cell differentiation suggests that targeting lysine demethylases could also provide novel drug targets to modulate T_H cell responses.[120] For example, GSK-J1, a chemical probe for the H3K27me3 demethylases, JMJD3 and UTX, was used to show that the catalytic activity of these enzymes has a critical role in lipopolysaccharide-induced proinflammatory cytokine production by human primary macrophages.[121] It is hoped that modulation of epigenetic modifiers may also have the ability to modulate the function of T_H cells.

SUMMARY

The factors controlling the epigenetic regulation of T_H cell responses are rapidly being defined. Repressive modifications are an important component of the epigenetic machinery that modulates T_H cell differentiation and function. Based on many studies, it appears that repressive modifications are found at high levels in naïve T_H cells and are lost on lineage-specific genes and accumulate on lineage-promiscuous genes during T_H cell differentiation (Figure 2). Importantly, the enzymes responsible—both methyltransferases and demethylases—have been shown to be amenable to modulation with small-molecule chemical inhibitors, offering hope for a new class of therapeutics to treat a wide range of diseases associated with dysregulated T_H cells.

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