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Review Article

Epigenetic Regulation of T-Helper Cell Differentiation

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Abstract

$CD4^+$ T helper (Th) cells are part of the adaptive immune system and are responsible for activating other immune cells, such as B cells, $CD8^+$ T cells, macrophages, mast cells, neutrophils, eosinophils, and basophils. Differentiation of $CD4^+$ T cells is influenced by cytokines and stimulation of the T cell receptor by different antigens. The pattern of cytokine secretion can be altered under specific conditions from one cell line to another, indicating that Th cells have plasticity. In fact, active and master regulators collaborate with transcription factors like signal transducers, activator transducers, and activators of transcription (STATs) in developing the differentiation process. The signals provided by cytokines activate specific transcription factors in each cell line. During this process, epigenetic modifications are actively involved. Epigenetics is defined as heritable alterations in the regulation of gene expression without any change in the DNA strand and includes DNA methylation, histone modification, and non-coding RNAs. The plasticity of $CD4^+$ T cells in differentiation to multiple subsets allows Th cells to exhibit the best immune response against the target microorganism. Failure to respond appropriately to multiple types of microorganisms can lead to disease. In this review, we have collected recent advances in understanding the role of epigenetic regulatory mechanisms in the differentiation of Th cells and, thereby, the commitment of $CD4^+$ T cells to a particular lineage to raise an appropriate response against a variety of microorganisms.

Keywords: $CD4^+$ T Cell; Cytokine; Epigenetics; Transcription Factor; T Cell Differentiation

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Introduction

$CD4^+$ T cells play a key role in the adaptive immune system through several mechanisms (1). The importance of $CD4^+$ T cells in patients with Human Immunodeficiency Virus (HIV) infection is well known, as these patients lack sufficient numbers of $CD4^+$ T cells and consequently develop a wide range of disorders. They also have an important role in the occurrence of autoimmune diseases, asthma and allergies, and possibly cancer. However, the T helper (Th) subsets are not from different lineages, and they can easily change the profile of their secretory products.

$CD4^+$ T cell differentiation represents a simple and tractable model for finding the basic principles of cellular specificity and gene regulation. These cells are divided into subgroups of Th1, Th2, Th9, Th17, Th22, and T regulatory (Treg) based on the nature of the antigen signal and the type of cytokines produced by the antigen-presenting cells (APCs)(2,3). Th1 and Th2 cells express specific chemokine and cytokine receptors; both contribute to the class-switching mechanism of immunoglobulins in B cells (4). Th9 cells play a role in allergies and autoimmunity, and also induce an immune response against melanoma and intestinal worms (5). Th17 cells are responsible for protecting against extracellular microbes and triggering autoimmune disease in mice (6). Interleukin (IL)-22, secreted from Th22 cells, participates in defense mechanisms and wound healing in the intestinal epithelium and skin tissue (7). While Treg cells are responsible for immune tolerance (8). Regulating the differentiation toward each subgroup is essential for regulating the immune system and protecting against various infections, as uncontrolled regulation of differentiation can lead to a variety of autoimmune and allergic diseases (9).

Although $CD4^+$ T subsets are stable, they also have plasticity among themselves, which is an inherent characteristic of T cell responses. In fact, $CD4^+$ T cells have the ability to perform different functions to respond against specific microorganisms and to alter their function in accordance with circumstances or different pathogens. A number of extracellular signals from the environment cause T cell reprogramming, but this occurs in the context of interactions between cytosolic signaling and epigenetic mechanisms (10). Hence, the question is, what factors both control the stability and make the cell phenotype plastic?

A bulk of studies have been conducted on the transacting factors and their role in the differentiation of $CD4^+$ T cells into each subset (11–13). Transcription factors are the major factors that determine the fate of $CD4^+$ T cell differentiation (4). These factors are controlled by epigenetic regulatory mechanisms, which ultimately lead to the expression or silencing of the target genes (14–16). Th9 and Th22 are new subgroups of effector T cells in which epigenetic mechanisms are significantly involved in their development. In this review, we describe epigenetic processes that regulate the differentiation of $CD4^+$ T cells toward Th1, Th2, Th17, and Treg cells by regulating gene transcription.

Epigenetic Mechanisms

The environment can affect the genome in two ways: one is the effect of the environment on the genome structure, which includes single-nucleotide mutations, and the other is the effect of environmental factors on gene expression without affecting the DNA strand (17). Those changes in gene expression are affected by environmental factors persisting for a long time in the absence of the inducing factors underlying the epigenetic changes (18). In fact, epigenetics refers to processes that alter gene expression patterns for a long time (19).

Epigenetic mechanisms appear early in the developmental process and are adapted to environmental stimuli throughout the life course, and can also be the source of many diseases later in life (20). Early epigenetic studies are from the early 20th century, which studied the mechanisms of embryonic developmental processes in the field of developmental biology (21). The most important epigenetic controlling components that affect the gene transcription process are chromatin arrangement modifiers, DNA methylation, histone modifications of the nucleus structure, and non-coding RNAs (ncRNAs)(22).

Chromatin Arrangement Modifiers

A variety of histone proteins, including H1, H2A, H3, and H4, act as compressors of the chromosomal DNAs (23). Histone tails can undergo post-translational modifications, such as acetyl-

ation, methylation, and phosphorylation. The histone protein gene has multiple copies that are initially expressed during the S phase of the cell cycle, while the histone variant gene is a single copy that is expressed during the whole cell cycle. Histone mRNA does not have a poly (A) tail, but histone variants have a poly(A) tail. One of the factors regulating chromatin structure in the cell is the replacement of histone proteins by their variants, which cause differences in expression or suppression of gene expression (24,25).

Histones surround DNA in chromatin and undergo acetylation, methylation, phosphorylation, and ADP-ribosylation in the N-terminal tail, among which acetylation is the most important modification (26). The most well-known histone-modifying enzymes are acetyltransferase factors (KATs) that transfer acetyl to lysine present in the histone and histone deacetylation factors (HDACs)(27). Transcriptional activation factors are activated by KATs, while transcriptional repression factors work by HDACs (28). Acetylation of histones reduces chromatin density and makes chromatin more accessible to binding proteins, eventually leading to increased transcription (29). Kinases modify the nucleosome by phosphorylating the histones, making DNA available to transcription factors (30). Methylation by methyltransferases in lysine and free arginine at H3 and H4 in the form of mono-, di-, and trimethylated culminates in different transcriptional results, depending on the position and type of histone. For example, methylation of H3K9 and H3K20 causes heterochromatin formation and transcriptional repression, whereas methylation of H3K4 and H3K36 activates transcription (31)(**Figure 1**). Hypermethylation of DNA promoter and coding sequences in tumor suppressor genes is involved in cancer progression (32). A study by O'Kane *et al.* in 2019 showed that histone post-translational modifications (PTMs) play a significant role in modulating the virulence of *Candida glabrata* (33).

DNA Methylation

DNA methylation in CpG islands is the most important DNA covalent alteration that causes gene silencing (34). DNA in eukaryotes is devoid of the CpG sequence, except for regions called CpG islands that are located in the promoter of genes. Mostly, methylation occurs in the early stages of growth, and as the cell progresses to the final stages, methylation levels decrease (35). DNA methylation is a stable, heritable, and reversible process that suppresses transcription. In mammals, there are three types of DNA methyltransferase (DNMT): DNMT1, DNMT3a, and DNMT3b that transfer the methyl group from the donor S-adenosine transferase to the carbon 5 of cytosine rings (36). In lymphocytes, especially naïve $CD4^+$ T cells, DNA methylation occurs when they differentiate into different types of T helper cells (37). Kobayashi *et al.* demonstrated that the regulation of DNA methylation in naïve T helper cells correlated with the clinical and pathophysiological status of minimal change nephrotic syndrome (MCNS), whereas in monocytes, the disease activity was not correlated with the level of DNA methylation (38).

Transcription factors may directly stimulate or suppress gene expression and may also influence transcription by taking up proteins that alter genes epigenetically. Some studies have shown that the 5aza-cytidine, a DNA methylation inhibitor, induces the production of IL-2 (39), and IFN-γ (40). By T cells that they were not previously able to produce. Studies also showed that $CD4^+$ T cells treated with HDACs increased the expression of both IFN-γ and Th2 cytokines (41,42). Puniya *et al.* reported that optimal levels of inputs, such as the composition and dosage of extracellular environment signals, can alter the phenotype of the $CD4^+$ T cells by increasing the activity of transcription factors related to a particular lineage (43).

Transcription Factor Networks that Drive Cell Differentiation

The nuclear factor of activated T cell (NFAT) and other transcription factors are activated in response to T cell receptor (TCR), and stimulatory molecules in $CD4^+$ naïve T cells induce interleukin (IL)-2 production, which induces signal transducer and activator of transcription five (STAT5), leading to initiation of the cell cycle. The development of Th1 cells is initiated by STAT1 in response to interferon (IFN)-γ and IL-27 produced by natural killer (NK) cells and antigen-presenting cells (APCs), respectively. Transcription factors, espe-

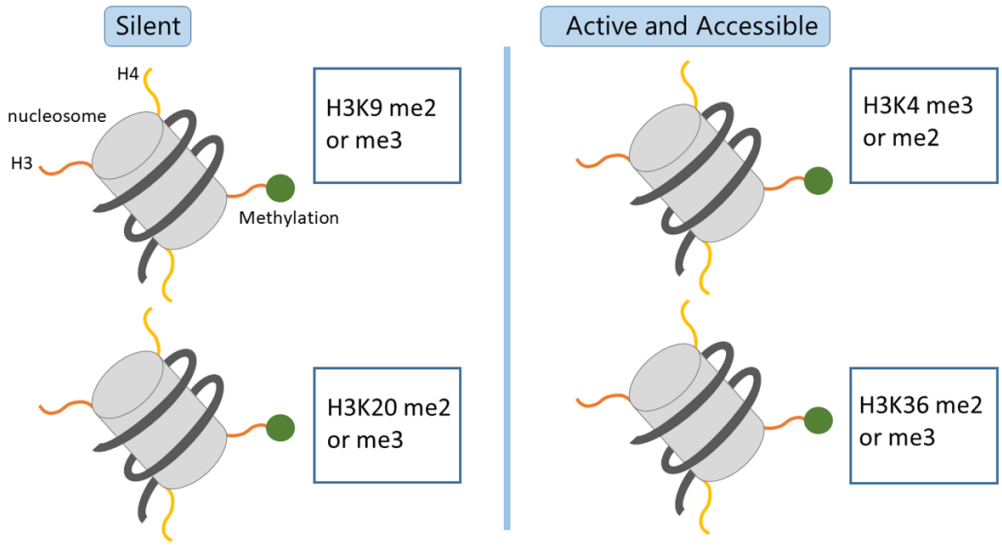

Figure 1. Chromatin and chromatin modifications. Acetylation (Ac) and methylation modifications to the tails of histones H3 (red line) and H4 (yellow line) in the promoter and enhancer regions of genes that are silent, active, and accessible. In this figure, for simplicity, modifications are exposed on only one of the two histone tails but may be present alone or in combination on one or both. Modification of H3K4 with one, two, and/or three (me1, me2, and/or me3) methyl groups is permissive. Modification of H3K9 and H3K27 with two and/or three (me2 and/or me3) methyl groups

cially STAT1, induce transcription factor T-bet, which induces IFN-γ and activates transcription factor H2.0-like homeobox (HLX) and runt-related transcription factor 3 (Runx3), resulting in suppression of GATA-binding protein 3 (GATA3) inhibitory effect. The binding of T-bet to Runx3 suppresses IL-4 transcription and leads to repression of Th2 differentiation (44–48).

During the differentiation of Th2 cells, STAT6 and GATA3 are activated by IL-4 and TCR-induced transcription factors, respectively. Notch signaling can also induce GATA3, which, in interaction with STAT6, activates the transcription of IL-4, IL-5, and IL-13 and blocks IFN-γ expression (49–51). In the presence of transforming growth factor (TGF)-β and IL-4, Th9 differentiation from naïve T cells occurs. Combination of these cytokines cause upregulation of IL-9 expression without inducing GATA3, and the Th2 cytokines, including IL-4, IL-5, and IL-13 (52). B cell activating transcription factor (BATF), interferon regulatory factor 4 (IRF4), and Purine-rich binding protein 1 (PU.1) are important in Th9 differentiation, but the Th9-specific transcription factor has not been clearly identified (53).

TGF-β inhibits differentiation of $CD4^+$ T cells into Th1 and Th2 cells and induces differentiation to Treg and Th17 cells by stimulating the expression of forkhead box P3 (Foxp3) and retinoic acid receptor-related orphan receptor gamma t (RORγt), respectively. In the absence of IL-6, Foxp3 inhibits RoRγt expression and inhibits differentiation to Th17 cells. Whereas, in the presence of IL-6, STAT3 prevents Foxp3 expression and increases differentiation to Th17 cells (54,55).

Th22 cells are differentiated in response to tumor necrosis factor (TNF)-β and IL-6. Based on studies, RORγt and Tbet transcription factors act as positive and negative regulators of Th22 differentiation, respectively (56).

The subtypes were first described by Mossman and Coffman (57), suggesting that Th1 cells expressing IFN-γ are responsible for protecting against intracellular viral and bacterial infection, while Th2 cells secrete IL-4, IL-5, and IL-13 cytokines and are involved in immune responses against extracellular infections and parasites (58). Th17 cells produce IL-17A, IL-17F, IL-21, IL-22, and IL-26 in humans, and participate in defense against extracellular bacteria and fungi, especially in the mucosal areas (59). However, Th22 cells produce IL-22 that is capable of driving lung inflammation in the presence of IL-17, while it is protective in the absence of IL-17 (60).

Despite the protective functions of Th cells, inadequate responses from Th1 and Th17 cells cause autoimmune disease, and Th2 cells are involved in the development of allergic responses (61). A combination of TCR and cytokines signaling activates transcription factors, leading to the differentiation of Th cells. In fact, precise control of gene expression is achieved through epigenetic processes, and gene expression can be altered in response to environmental changes. During the differentiation of Th cells, epigenetic modifications activate related transcription factors and suppress the expression of others (62).

Epigenetic Control of $CD4^+$ T Cell Differentiation

Th1 Subset

The *IFNG* gene is not in the same group as other co-expressing cytokine genes. In invertebrates, the closest upstream neighboring genes of *IFNG* are *IL-22* and *IL-26*, which are expressed by Th17 cells (63,64). Several regulatory elements and conserved non-coding sequences (CNS) have been identified in mice up to 70-60 kilobases (kb) upstream and downstream of the *IFNG* locus, including enhancers in CNS34, CNS22, CNS6, CNS+18-20, CNS22, and CNS+46 (65). Recent genomic analyses in the hypersensitive site and histone modifications in human $CD4^+$ T cells indicate that similar regulatory elements are also present in the human *IFNG* locus (66). The activated naïve $CD4^+$ T cells produce a small amount of IFN-γ, indicating that the *IFNG* locus is in a ready state. Naïve T cell DNA is demethylated in the mouse *IFNG* promoter, CNS-34, CNS-22, CNS+29, and CNS+46, and CNS-34 and CNS-22 exhibit low levels of H3K4 demethylation and H4 acetylation (65,67,68). In contrast, there is a moderate level of H3K27 repressive trimethylation between *IFNG* and CNS+18-2020, from CNs+29 to CNs+46 and adjacent to CNs-22. Across the *IFNG* locus, bivalent histone modifications prepare it for expression on or off. In differentiation to Th1, increased H3K4 demethylation, H3, and H4 acetylation, the attainment of DNaseI hypersensitive site by regulatory elements in the *IFNG* locus and loss of H3K27 repressive trimethylation occur throughout the locus (69). However, H3K9 repressive demethylation is induced at specific sites of the *IFNG* locus in Th1 cells, which prevents the initiation of incorrect transcription (70). In contrast, when the cell differentiates into Th2, the permissible histone modifications are lost, H3K27 repressive trimethylation and DNA CpG methylation also increase throughout the locus, and NFAT loses its ability to bind to the *IFNG* promoter (68,71).

STAT1 enhances *IFNG* transcription through T-bet expression. In addition, STAT5 directly promotes the transcription of *IFNG* through binding to the *IFNG* promoter and *CNS+18-20*, thereby facilitating histone acetylation, chromatin modifications, and T-bet binding to the *IFNG* promoter (72). The binding of STAT4 to the IFNG promoter and other regulatory elements, including CNS22, leads to epigenetic changes that permit gene expression (45). T-bet directly activates *IFNG* transcription and has additional effects. It specifically binds to the *IFNG* promoter and many other enhancers **(Figure 2)**, induces the expression of H2.0-Like Homeobox (HLX), and runt-related transcription factor 3 (RUNX3). Through these transcription factors, it binds to the *IFNG* promoter, and RUNX3 binds to the *IL-4* silencer, preventing GATA3 expression and its function (73). T-bet binds to the *IFNG* promoter even when the DNA is methylated and employs HAT instead of the HDAC complex (74). Moreover, Usui *et al.* recently showed that *IFNG* promoter accessibility, as detected by histone acetylation and deoxyribonuclease I hypersensitivity, and the negative effect of GATA3 on Th1 differentiation arise from its ability to suppress STAT4 levels.

Th2 Subset

Changes in the chromatin structure of the *IL-4* and *IL-13* genes cause differentiation of naïve T cells into Th1 or Th2 subsets (75,76). In mice, the Th2-related locus contains the *IL-4, IL-5, IL-13,* and *Rad50* genes. The promoters and regulatory elements of the Th2-related locus are regulated through the identification of DNaseI hypersensitive sites (HS), histone modifications, and DNA methylation, as well as through computational identification of CNS (77). At the Th2-related locus in mice, IL-4 transcription increases through mapping regulatory elements to the HSI, HSII at the second intron of *IL-4*, DNaseI hypersensitive site vA (HSVA), HSV located at the 3' end of *IL-*

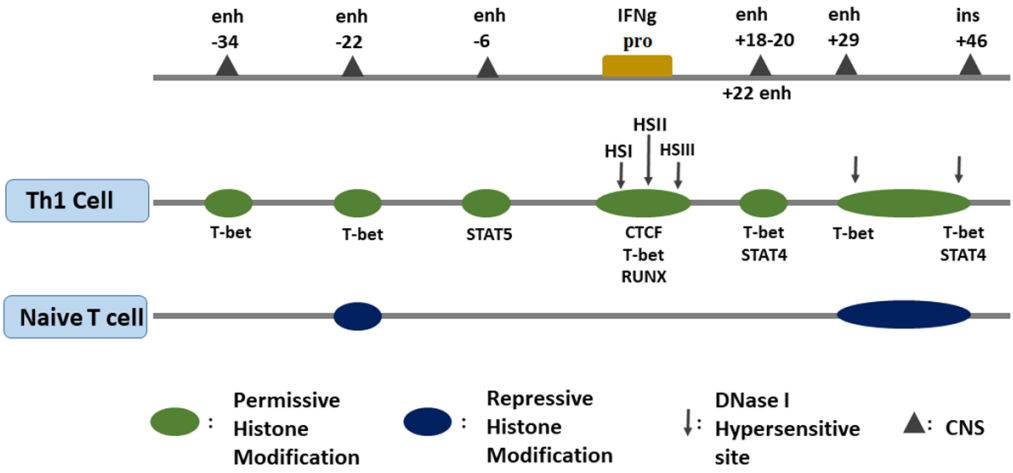

Figure 2. The IFNG locus in mouse naïve and T helper 1 cells. DNaseI hypersensitive sites at conserved non-coding sequence -34 (CNS-34) and near CNS+46 in naïve mouse CD4+ T cells have low levels of permissive histone modifications (such as acetylated H3, acetylated H4 and dimethylated and/or trimethylated H3K4) at IFNG (interferon-γ), CNS-22 and CNS-34 and repressive trimethylated H3K27 (blue regions) at the 3′ end of the locus. At CNS-34 and CNS-22, the IFNG promoter (pro), CNS+29, and CNS+46 DNA is demethylated. In T helper 1 (Th1) differentiation, hypersensitive site I (HSI), HSII and HSIII, DNaseI hypersensitive sites at several CNS enhancers sites, and high levels of permissive histone modifications (green regions) are developed, but trimethylated H3K27 is lost. The function of specific elements, such as promoters (pro), enhancers (enh), and insulators (ins), are indicated, as are the binding sites for the lineage-restricted transcription factors CCCTC-binding factor (CTCF), runt-related transcription factor 3 (RUNX3), signal transducer and activator of transcription 4 (STAT4), STAT5 and T-bet.

4, and DNaseI hypersensitive site s1 (Hss1), HSs2 located between *IL-4, IL-13*, and Th2-related cytokine locus control region (LCR), which includes Rad50 hypersensitive site 4 (RHS4), RHS5, RHS6,, and RHS7 (49). IL-13 expression is also increased by regulatory elements on the CNS1, Th2-related cytokine LCR, and HSI that move to the CG-rich element (CGRE) upstream of the *IL-13* promoter. Most of the Th2-related locus promoters and enhancers are directly targeted by NFAT and other induced transcription factors. For example, STAT6 binds to the *IL-4, IL-13*, HSVA promoter, as well as RHS6 and RHS7, and GATA3 binds to the *IL-5, IL-13*, HSVA, RHS7 promoter and HIS-CGRE regions in *IL-13* (78). Schieck *et al.* reported that polymorphism in the Th2-related LCR, like RHS7 in mice, affects DNA methylation and gene expression within 5q31 and even total serum IgE levels in the population (79). Naïve $CD4^+$ T cells express low levels of GATA3 and T-bet factors and produce low mRNA levels of *IL-4, IL-13*, and *IL-5* (80,81). In these cells, the Th2-related locus lacks HSI and histone changes **(Figure 3)**.

The expression of Th2-related cytokines is probably inhibited by high methylation of CpG in their promoter, CNS1, CNS2, and Th2-related cytokine LCR (82–86). These epigenetic states cause binding of TCR-induced transcription factors and expression of Th2-related cytokines (49,58). Following activation of naïve $CD4^+$ T cells by NFAT and other TCR-induced transcription factors, permissible histone modifications in the Th2-related cytokine locus occur within the first 24-48 hours (87). These transcription factors also induce IL-2 expression, which activates STAT5 and induces chromatin changes in intron 2 of the *IL-4* promoter to promote Th2 differentiation (88). An active locus of Th2 cells has a hypersensitive site with new sites in the *IL-4*, *IL-5*, and *IL-13* promoters. H3 and H4 acetylation and H3K4 demethylation also occur in these elements. H3K21 repressive trimethylation is lost throughout the locus, and HS demethylation is initiated (89). GATA3 is required to induce epigenetic changes in Th2 cells. This can be conducted directly through the HATs or indirectly via histone H3K4 methyltransferases (90). STAT6 can also facilitate the differentiation of Th2 by binding to several sites in the Th2-related cytokine locus (78)**(Figure 3)**. Patrick E. Fields *et al.* showed that histone acetylation, which occurs in *IL-4* loci during Th2 differentiation, is locus and lineage-specific and is maintained by the transcription factor GATA3 in

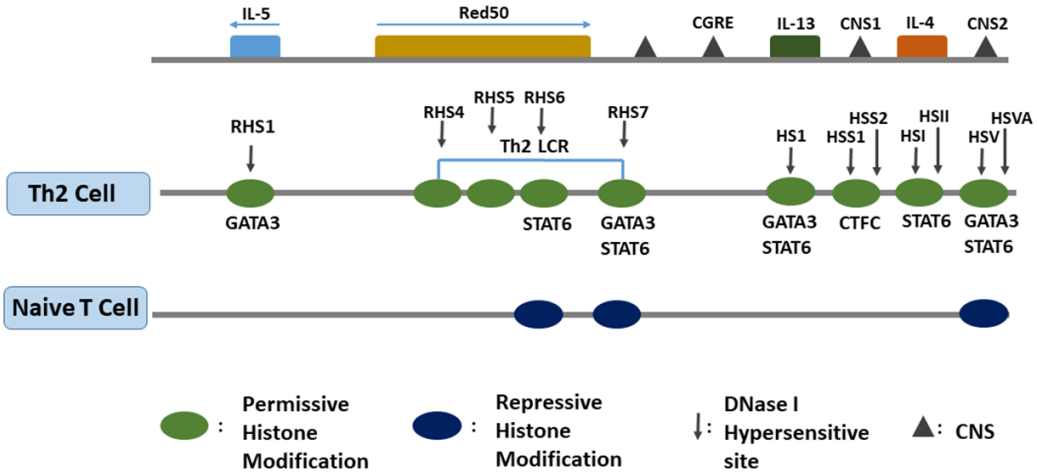

Figure 3. The T helper 2 cytokine locus in mouse T cells. DNaseI hypersensitive sites at hypersensitive site s3 (Hss3), HSIV, the 5′end of the Rad50 gene at Rad50 hypersensitive site 2 (RHS2) and RHS3, and perhaps at RHS6 in the locus control region (LCR) of the T helper 2 (Th2)-cytokine locus are present in naïve CD4+ T cells. In Th2 cells, DNaseI hypersensitive sites and substantial levels of permissive histone modifications (such as acetylated H3, acetylated H4, and dimethylated and/ or trimethylated H3K4; green regions) are developed at the promoters and enhancers of IL4 (interleukin-4), IL13 and IL5. The binding sites for the lineage-restricted transcription factors MAF, CCCTC-binding factor (CTCF), GATA-binding protein 3 (GATA3), runt-related transcription factor 3 (RUNX3), signal transducer, and STAT6 are also shown. In the Th2-cytokine locus, DNaseI hypersensitive sites are mentioned by their commonly used names, in which sites in or near IL4 are indicated as HS followed by a Roman numeral (for instance, HSIV), sites between IL4 and IL13 are indicated as Hss followed by a number (for instance, Hss1) sites in or upstream of IL13 are indicated as HS followed by a number (for instance, HS1), and sites in or near Rad50 are indicated as RHS followed by a number (for instance, RHS6).

a STAT-dependent manner (91).

Th17 Subset

Little data is available on the regulatory mechanism and epigenetic process of Th17 differentiation. IL-17A and IL-17F are expressed simultaneously in Th17 cells, and the genes encoding them are colocalized in mammals, suggesting that they are regulated by common regulatory elements. In mice, eight regulatory elements have been described in the *IL-17* locus (92)**(Figure 4)**. In these eight elements, similar to the *IL-17A* and *IL-17F* promoter, H3 acetylation is increased, which is more than naïve CD4 T cell, Th1, and Th2 (93). Binding of STAT3 to the *IL-17a* and *IL-17f* promoters and H3 acetylation of it leads to Th17 differentiation (54,94). Of course, RoRγt and RoRα do not bind to this promoter, but they bind to CNS2 (a ROR-dependent enhancer) upstream of IL-17A **(Figure 4)**. Genes encoding other Th17 cytokines, such as IL-21, IL-22, and IL-26, are located on different chromosomes and are close to genes expressed by Th1, not Th17 (95). Sin *et al.* reported that activating transcription factor 7 interacting protein (ATF7ip), as a critical regulator of Th17 differentiation, inhibited *IL-2* gene expression by deposition of the repressive histone mark H3K9me3 in the *IL-2-IL-21* intergenic region (96). Xiunan Wang *et al.* recently showed that JQ1 (the bromodomain inhibitor) suppresses the response of Th17 cells by impairing histone acetyltransferase p300 (bromodomain-containing protein) of RORγt (97).

Treg Cells

In mice and humans, there are distinct regions of the *FOXP3* locus showing DNA methylation patterns and specific histone modifications that differ between Treg cells and common T cells. Sequence analysis has shown that there are three conserved non-coding regions in *FOXP3* locus, all of which undergo epigenetic changes and are involved in the regulation of *FOXP3* transcription (11).

FOXP3 Promoter

The *FOXP3* promoter, located 6.5 kb above the first *FOXP3* coding exon, is a classic TATA and CAAT box promoter that is activated in response to TCR signaling through the binding of NFAT

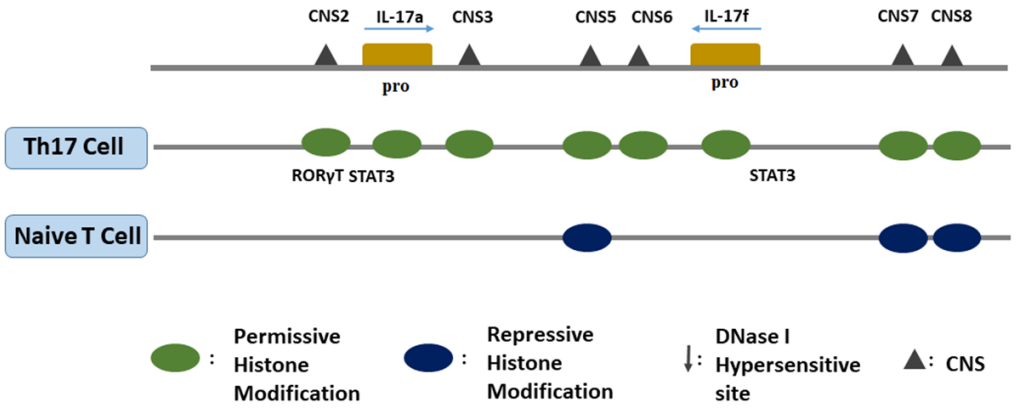

Figure 4. The T helper 17 cytokine locus in mouse T cells. Weak permissive histone H3 acetylation is present in naïve mouse CD4+ T cells at conserved non-coding sequence 5 (CNS5), CNS7, and CNS8. By contrast, in Th17 cells, higher levels of H3 acetylation (green regions) are exhibited at these regions, at other CNS in this region, and at the IL-17A (interleukin-17A) and IL-17F promoters. In TH17 cells, the IL-17A and IL-17F promoters are binding by signal transducer and activator of transcription 3 (STAT3), and it has been shown that when retinoic-acid-receptor-related orphan receptor-γt (RoRγt) bind to CNS2, when it is overexpressed.

and AP1 (98). Treg and other T cell subsets show differences in epigenetic changes in both humans and mice. The CpG motif in the *FOXP3* promoter is almost completely demethylated in Treg cells, whereas they are methylated in naïve conventional $CD4^+$ T cells (99). In addition, the *FOXP3* promoter in Treg cells exhibits highly acetylated histones in comparison to the conventional T cells, indicating that the *FOXP3* promoter is more accessible in Treg cells and is overexpressed (100) **(Figure 5)**.

TGFβ Sensor

The second conserved non-coding region in the *FOXP3* locus is known to be a TGFβ-sensitive element that contains binding sites for NFAT and the TGFβ-induced transcription factor mothers against decapentaplegic homologue 3 (SMAD). Chromatin is also accessible in this region in cells expressing Foxp3 **(Figure 5)**. In both natural and TGFβ-induced Treg cells, elevated levels of acetylated histone H4 were observed in the regions containing TGFβ-sensitive elements (101). In addition, remodeling of the TGFβ-induced chromatin structure in this region may affect access to the upstream *FOXP3* promoter; thus, the promoter demethylation rate is slightly increased in TGFβ-treated mouse T cells (99).

Treg Cell-Specific Demethylated Region (TSDR)

Significant differences have been observed for the methylation pattern in the *FOXP3* locus in the third CpG-rich conserved region. This region is completely demethylated in Treg cells and methylated in common T cells (102). In addition, acetylated histones H3 and H4 and trimethylated lysine 4 in histone H3 (H3K4me3) were accumulated in TSDR. TSDR has a booster activity that is significantly reduced after methylation (103), and the cAMP response element binding protein (CREB) transcription factor is bound to TSDR when this region is demethylated. Thus, TSDR demethylation is consistent with Foxp3 expression stability (as in natural Treg cells), whereas T cells expressing Foxp3 only transiently (TGFβ-induced Treg cells and recently activated, commonly human T cells) have methylated TSDR (104) **(Figure 5)**. Liu *et al.* demonstrated that alteration of DNMT1 in T cells affects hepatocellular carcinoma (HCC) growth by altering the methylation of Foxp3 both in the promoter and CpG regions (105). Another study by Cao and colleagues revealed that epigenetic modifications are also an essential part of the upregulation of Foxp3 from naïve $CD4^+$ T cells. Ying Shao *et al.* showed that histone modification enzymes are more downregulated in metabolic diseases and regulatory T cell (Treg) differentiation (106).

Application of Epigenetics in Disease Treatment

Uncontrolled T-cell responses are characteris-

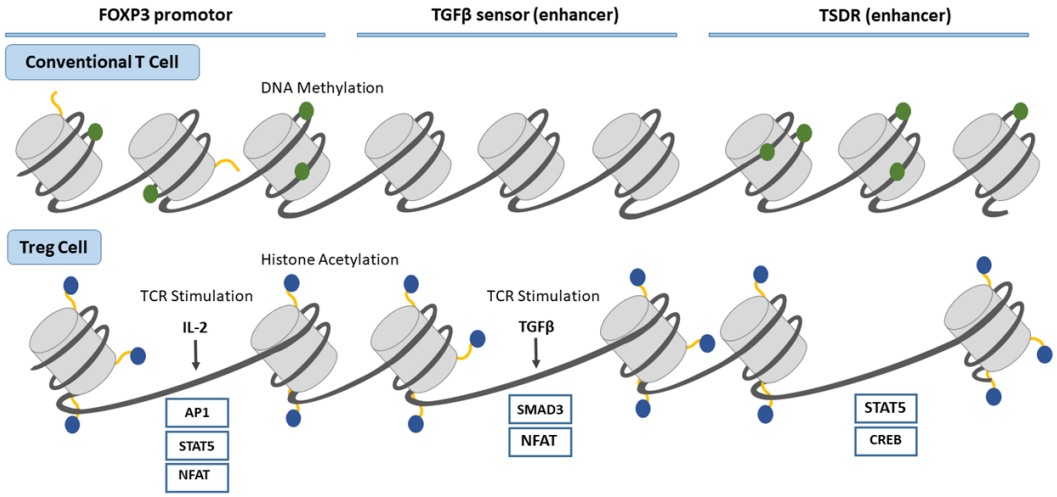

Figure 5. The epigenetic control of FOXP3 locus. Three conserved non-coding regions that undergo epigenetic modifications and are involved in regulating FOXP3 transcription are shown. Histone acetylation and DNA methylation are epigenetic modifications in these three regulatory regions, which are depicted for Foxp3 conventional T cells and natural Treg cells (natural Treg cells show a stable Foxp3+ phenotype). Note that CpG motifs are not consistent in the TGFβ sensor region. Permissive histone modifications and DNA demethylation induce an open chromatin conformation that allows transcription factors to bind to regulatory sites and thereby induce and stabilize the expression of FOXP3. Upstream signaling pathways that affect these regions follow the activation like activator protein 1 (AP1); interleukin-2 (IL2); cyclic-AMP-responsive-element-binding protein (CREB); nuclear factor of activated T cells (NFAT); mothers against decapentaplegic homologue 3 (SMAD3) and signal transducer and activator of transcription 5 (STAT5) are depicted.

tic of several inflammatory diseases, including inflammatory bowel disease (IBD), rheumatoid arthritis (RA), diabetes, asthma, and allergies (107). Th1 cells are associated with several inflammatory diseases, including IBD, RA, and diabetes. On the other hand, overexpression of type 2 cytokines by Th2 cells can lead to pathological conditions, including asthma and allergies (108,109). In addition, uncontrolled cellular responses of Th17 and induced Treg (iTreg) have also been seen in a wide range of inflammatory conditions, including multiple sclerosis, RA, and IBD (110). Therefore, a better understanding of the molecular mechanisms that control the differentiation of Th-cell subsets could provide novel therapeutic targets for the treatment of this wide range of inflammatory diseases.

The etiopathogenesis of cancer has been suggested to be underlying genetic variations and epigenetic dysregulations that may play an important role in the initiation and perpetuation of the disease. Epigenetic therapy has been proposed as one of the potential cancer treatment approaches (111). Unlike genetic mutations, epigenetic changes are reversible. The most common anti-cancer drugs that cause epigenetic changes in tumor cells are DNA methylation inhibitors and HDAC inhibitors (112). The inclusion of epigenetics in the National Institutes of Health (NIH) map has highlighted the need for research on both epigenetic mechanisms in oncogenesis and epigenetic therapies. Many compounds have been discovered that can alter DNA methylation patterns and histone modifications, and some of them are currently being tested in clinical trials (113)(**Table 1**). 5-azacytidine (5-aza-CR) and 5-aza-2-deoxycytidine (5-aza-CdR), which were initially developed as cytotoxic agents, are powerful inhibitors of DNA methylation and induce gene expression and differentiation in cultured cells (114,115). Also, HDAC inhibitors can induce differentiation, growth arrest, and/or apoptosis in transformed cells in culture as well as in tumors (116). The US Food and Drug Administration (FDA) has approved three targeted epigenetic agents for oncology, namely Vidaza, Dacogen, and Zolinza, and many others that are in clinical and pre-clinical development (117).

Conclusion

Epigenetic regulatory mechanisms are involved in gene expression, controlling immune cells' activation, differentiation, and effector function. During the immune response, induction and ter-

Table 1. Epigenetic drugs

Target	Drug	Status
DNMT inhibitor	5-Azacytidine	FDA approved, 2004
	5-Aza-2_-deoxycytidine	FDA approved, 2006
	EGCG	Clinical trials (Phase I)
	Antisense oligomers	Clinical trials (Phase I)
HDAC inhibitor	SAHA	FDA approved
	Depsipeptide	Clinical trials (Phase I/II)
	Valproic acid	Clinical trials (Phase I/II)
	Phenylbutyric acid	Clinical trials (Phase I/II)

mination of immune cell activity must be monitored precisely to prevent pathological inflammation. The discovery of epigenetic regulatory factors that modulate gene expression during the differentiation of $CD4^+$ T cell subtypes can lead to the identification of new immune checkpoints and their therapeutic implications. In addition, the development of new therapeutic approaches to restore the epigenome of immune cells in order to correct the proliferation and differentiation of T cells in tumors or autoimmune diseases could be feasible with future clarifications.

Conflict of Interest

The authors declare to have no conflict of interest.

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