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Epigenetics and T-cell immunity

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Abstract

Epigenetic mechanisms including DNA methylation and histone modifications are critically involved in immune responses. Antigen stimulation along with a specific cytokine milieu drives helper T-cell differentiation into specific subsets with distinct functional capacities. This process occurs by inducing chromatin remodeling and altering transcriptional accessibility of key cytokine genes such as IFN-γ, IL-4 and IL-17. These epigenetic changes, by definition, are carried over throughout cell division to ensure selective survival of a cell lineage. Over the past decade, a growing body of literature mechanistically uncovered the central role for epigenetic regulation in immunity. In this review, we focus on epigenetics in T helper cell differentiation, regulatory T-cell function, and IL-2 production.

Keywords: *Epigenetics, gene regulation, T-cell immunity, T-cell function, DNA methylation*

Introduction

The immune response has evolved to efficiently control and eliminate potentially dangerous infectious agents that are encountered on a daily basis. The response must be both robust, to rapidly neutralize the threat, and highly specific, to minimize damage to the host. One way this occurs is by rapid expansion of clonal populations of immune cells specific for antigens present on the pathogen, a process that requires heritable mechanisms to maintain specific cell lineages during proliferation and clonal expansion. Epigenetic regulation is one mechanism that is used extensively by the immune system to mount a rapid, robust, specific immune response against a pathogenic organism. Over the past decade, epigenetic regulation, mainly through DNA methylation and histone modifications, has been increasingly recognized as central in the differentiation and function of helper T-cell subsets. This review focuses on the mechanisms and roles of these epigenetic processes in regulating helper T-cell differentiation during a normal immune response.

Epigenetics and gene regulation

Epigenetics refers to heritable changes in genes, often altering their expression, that occur without changes in the DNA sequence. This provides a means to activate transcription of genes necessary for a particular cell type or function and to silence genes that are not needed. It is important for a number of vital biological processes, such as tissue differentiation [1], imprinting [2], X chromosome inactivation [3] and suppression of transcriptional "noise" and foreign DNA [4]. Genes can be regulated epigenetically in several ways. These include DNA methylation, histone modification and RNA interference [5]. Normal immune responses have been shown to employ both DNA methylation and histone modifications and these mechanisms are discussed below.

DNA methylation refers to addition of a methyl group from S-adenosylmethionine to the Cytosine-Guanosine of a CG dinucleotide at the fifth carbon in the cytosine ring [6]. This can be mediated by DNA methyltransferases 1, 3a or 3b. In general, DNA

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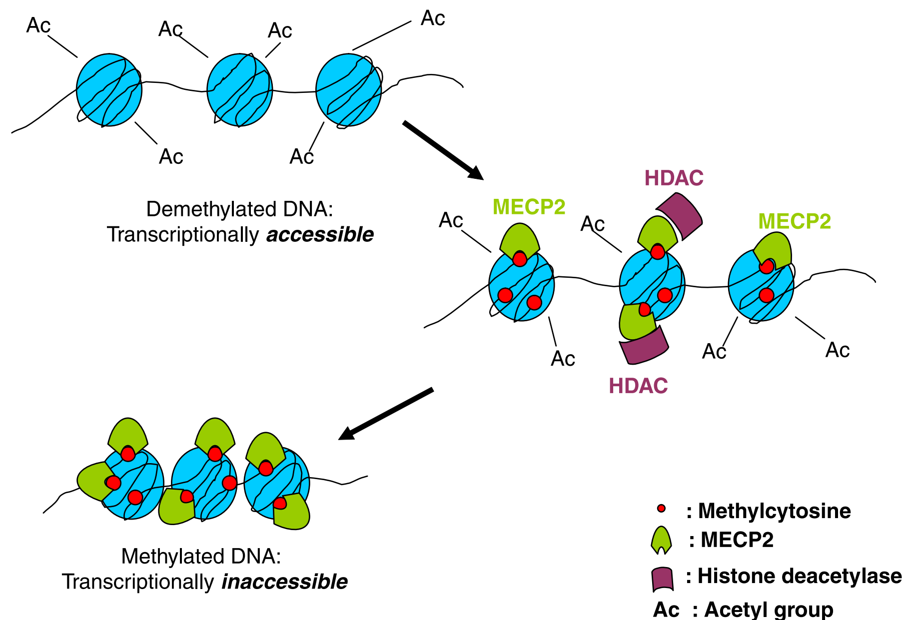

Figure 1. DNA methylation suppresses gene expression. DNA demethylation and histone acetylation promotes gene expression via inducing chromatin configuration that is accessible to transcriptional machineries. When DNA is methylated, methylcytosine residues bind transcriptional repressors such as methylcytosine binding protein 2. MECP2 recruits histone deacetylases, which by removing acetyl groups from histone tails, increase the charge attraction between the chromatin and histone proteins. This results in dense chromatin configuration that is not accessible for transcription.

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methyltransferase 1 (DNMT1) is involved in maintaining DNA methylation patterns during cell division [7–9], whereas DNA methyltransferase 3a and 3b (DNMT3A and DNMT3B) are responsible for determining the pattern and extent of DNA methylation during fetal development, and hence are known as *de novo* DNA methyltransferases [10]. CG dinucleotides are concentrated in or around transcription initiation sites of methylation sensitive genes. In some methylation sensitive genes, CG dinucleotides are found in regions referred to as CpG islands, defined as at least 200 bp of DNA with a C + G content of at least 50% and a CG frequency of at least 0.6 [11]. In general, most CG sites are methylated, with the exception of CG sites in promoters of transcriptionally active genes. Heavily methylated genes are generally silenced, while hypomethylation in methylation sensitive genes induces gene transcription [12]. Methylation-sensitive gene repression can occur by more than one mechanism. Transcription inhibition can occur through stearic hindrance of transcription factor binding to DNA, caused by the addition of bulky methyl groups [13]. Inhibition can also occur through binding of methylcytosine binding proteins to methylated DNA, which causes an increase in chromatin density, making the initiation site inaccessible to the transcription machinery [13]. In fact, the ability of methylcytosine binding proteins such as MECP2 to repress transcription has been directly linked to recruitment of histone deacetylases that function in chromatin condensation ([14,15]; Figure 1).

Chromatin density can be altered by histones, small nuclear proteins that form the central core of nucleosomes. Nucleosomes form the basic unit of chromatin structure and consist of ~147 bp of DNA wrapped nearly twice around a histone octamer. The octomer consists of two copies of each histone protein H2A, H2B, H3 and H4 [16]. The organization of nuclear DNA into higher chromatin structures allows the large amount of DNA to condense into chromosomes as well as performing a transcriptional regulation function by altering access to the DNA by the transcription machinery [17]. Histone tails are protein domains that protrude beyond the surface of the chromatin polymer and these domains can be extensively modified post-translationally [18]. Modifications including acetylation, phosphorylation, methylation, ubiquitylation and sumoylation have been demonstrated [17,19]. Certain histone modifications promote transcription activation while others promote gene silencing. Histone acetylation, which can take place on a number of lysine residues within any of the four histone proteins, is generally associated with increases in transcription [19]. This is thought to be primarily an effect of weakening of the charge attraction between histone and DNA. In contrast, histone deacetylation, mediated by histone deacetylases, strengthens the DNA/histone charge attraction, thus favoring a chromatin configuration that is not accessible for transcription [20]. On the other hand, histone methylation, which is a posttranslational modification that mainly affects lysine residues

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within histones H3 and H4, has variable effects on transcription. For example the methylation of lysine 4 of histone H3 (H3K4) results in transcriptional activation, whereas the methylation of lysine 9 (H3K9) is generally associated with transcriptional repression [19].

Helper T-cell subsets and function

Activation of naïve CD4 + helper T cells via the T-cell receptor (TCR) and MHC II: peptide complex induces a rapid T-cell differentiation process. Differentiated helper T cells are classified according to the type of cytokines they produce into Th1, Th2, or the more recently recognized Th17 lineage. Both Th1 and Th2 have been extensively studied since the initial description of helper T-cell subsets [21]. The differentiation into either Th1 or Th2 is determined by the type of the infectious microbe recognized by the immune system of the host.

Th1 is involved in immune responses against intracellular pathogens. Indeed, the delayed type hypersensitivity reaction for certain mycobacterial infections is a classical example for a Th1 immune response [22,23]. On the other hand, the Th2 helper T-cell subset is critical in immune responses to extracelluar pathogens such as parasitic helminthes, and in infections in which B-cell stimulation is important [23]. A number of human immune-mediated diseases are characterized by an abnormal Th1 or Th2 T-cell response. For example, asthma and allergies are diseases characterized by abnormally high Th2 activation [24]. In contrast an aberrant Th1 response characterized by the predominance of interferon gamma (IFN-γ), occurs in rheumatoid arthritis [25]. Recent data, however, suggest a prominent role for the IL-17 producing Th17 cells in inducing the joint damage in rheumatoid arthritis [26].

Distinction between the helper T cell subsets is based on the type of cytokines produced. The predominant effector cytokine produced by the Th1 subset is IFN-γ, while IL-4 is predominant for the Th2 subset [23]. IFN-γ is primarily involved in activating and augmenting the microbicidal effects of macrophages and inducing production of opsinizing immunoglobulins that enhance phagocytic elimination of pathogenic microbes. In the presence of IL-2, IFN-γ also activates cytotoxic CD8 + T-cell [23]. The main cytokine produced by Th2 T helper cells, IL-4, is a principle inducer of B-cell activation. IL-4 induces immunoglobulin class switch to IgE, which binds irreversibly to the IgE receptor on mast cells and by so doing activates them. Another Th2 cytokine is IL-5 which has a principle role in activating eosinophils [23].

Therefore, the pattern of cytokines produced by helper T-cell subsets explains the functional diversity between the Th1 and Th2 immune responses.

Helper T-cell differentiation

Differentiation of helper T cells into either Th1 or Th2 is, at least in part, determined by the cytokine milieu (environment) where T-cell stimulation via the TCR and MHC II: peptide complex is taking place. IL-12, secreted by activated dendritic cells and macrophages, mediates helper T-cell differentiation into a Th1 cell (Figure 2). Indeed, IL-12 receptor is expressed on undifferentiated naïve CD4 + T cells as well as Th1 cells, but a functional IL-12 receptor is eliminated in Th2 cells [27]. IL-12 deficient mice also show impaired IFN-γ production and Th1 differentiation [28]. On the other hand, IL-4 causes differentiation of naïve CD4 + T cells into Th2 cells, and IL-4 −/− mice are deficient in Th2 cell differentiation ([29]; Figure 2).

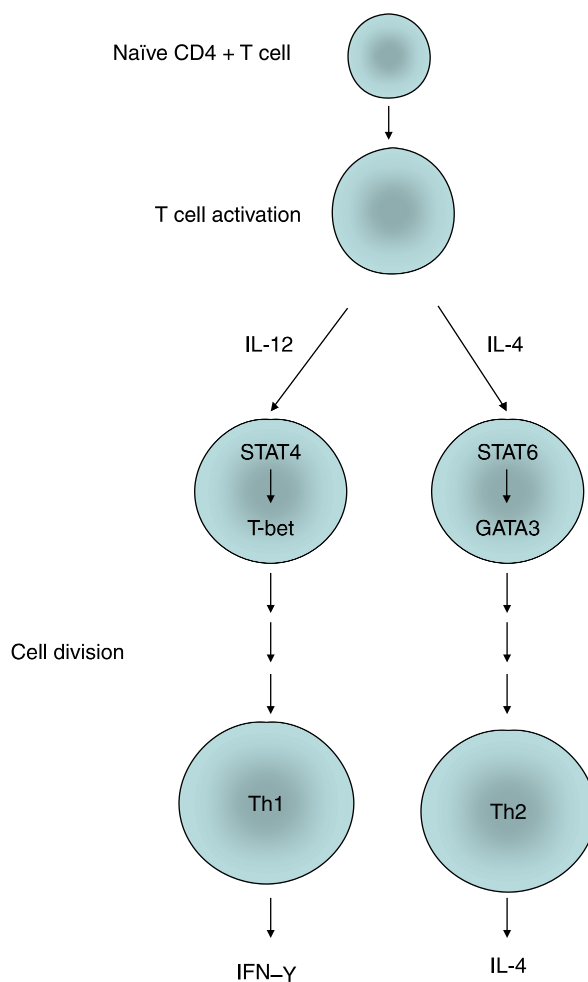

Figure 2. Helper T-cell differentiation. Th1 and Th2 cells differentiate from a common precursor naïve CD4 + T-cell. The differentiation process depends on the cytokine environment that is determined largely by the type of antigen stimulation. IL-12 signals through the STAT4 signaling pathway to promote expression of the transcription factor T-bet that mediates Th1 differentiation. In the presence of IL-4, the transcription factor GATA3 is induced via STAT6 signaling resulting in Th2 cell differentiation. Th1 differentiated cells produce IFN-γ, while the main cytokine produced by Th2 cells is IL-4.

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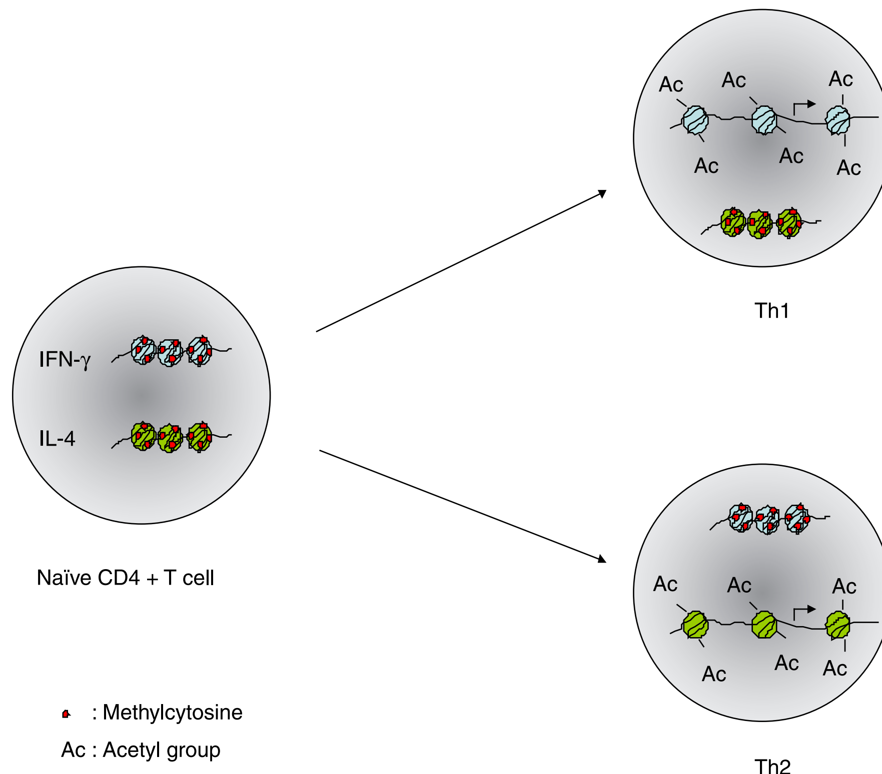

Figure 3. Epigenetic changes regulate helper T-cell differentiation. In naïve CD4 + T cells, both IFN-γ and IL-4 genes are DNA methylated and histone deacetylated resulting in chromatin remodeling that is transcriptionally non-permissive. In Th1 conditions, the IFN-γ locus demethylates and becomes acetylated, while the IL-4 locus is methylated and deacetylated, thus inducing chromatin remodeling that allows for IFN-γ but not IL-4 expression. On the other hand, in Th2 conditions, the IFN-γ locus is heavily methylated and deacetylated, while the IL-4 locus demethylates and is acetylated, thus IL-4 is expressed while IFN-γ expression is silenced. The differential effects on methylation and acetylation of IFN-γ and IL-4 loci in Th1 and Th2 cells is largely determined by the transcription factors T-bet and GATA3, which promote Th1 and Th2 differentiation, respectively.

This polarization of CD4 + T cells by the cytokine environment is mediated by the activation of signaling pathways and transcription factors distinct for either Th1 or Th2 subsets. IL-4 activation of STAT6 signaling and the expression of the transcription factor GATA3 ensures differentiation into Th2 [30,31]. Indeed, T cells from STAT6 knock out mice are incapable of differentiating into Th2 cells in response to IL-4 [32]. Similarly, GATA3 has been shown to be necessary for Th2 cytokine expression as antisense GATA3 inhibited expression of all Th2 cytokine genes in the Th2 clone D10 [30]. On the other hand, differentiation of naïve helper T cells into the IFN-γ-producing Th1 cells in the presence of IL-12 is mediated through the activation of STAT4 signaling and the transcription factor T-bet [33,34]. Differentiation into Th1 cells is impaired in STAT4 or T-bet deficient mice [35,36]. Curiously, however, T-bet is not involved in control of IFN-γ production in CD8 + T cells [36].

A negative feed back mechanism to ensure Th1 and Th2 polarization also exists. IL-4 down regulates IFN-γ production by inducing STAT6 target gene transcripts such as GATA3 which inhibits IFN-γ expression [37]. In contrast, the IL-12 induced transcription factor T-bet down regulates Th2 cytokine expression [37]. A more recent study revealed the role of T-bet in inducing the transcription factor Runx3, which cooperates with T-bet to activate IFN-γ and silence IL-4 in Th1 cells [38]. Therefore, the Th1 transcription factor T-bet and the Th2 transcription factor GATA3 downregulate Th2 and Th1 cytokine expression, respectively.

Epigenetics in helper T-cell differentiation

Th1 and Th2 differentiation

T-bet and GATA3 can induce chromatin remodeling of IFN-γ and IL4/IL5 genes, respectively. The chief effector cytokines in Th1 and Th2 cells, IFN-γ and IL-4, are both expressed in a cell-cycle-dependent manner [39]. The expression of IFN-γ increases with each successive cell cycle, and the expression of IL-4 requires at least three cell divisions. Once helper T cells are terminally differentiated, however, the production of effector cytokines becomes cell cycle independent [39]. The observation that differentiation of naïve CD4 + T cells into either Th1 or Th2 is linked to cell division, suggests a role for epigenetic

mechanisms and chromatin remodeling in the differentiation process (Figure 3). Indeed, stimulation of naïve helper T cells in the presence of either the DNA methylation inhibitor 5-azadeoxycytidine or the histone deacetylase inhibitor sodium butyrate, results in marked increase in the numbers of IFN-γ and IL-4 expressing cells [39]. In addition, expression of Th2 cytokines in T cells from Dnmt1 deficient mice is substantially increased, supporting the notion that DNA methylation plays a major role in Th2 cytokine repression [40].

Chromatin modifications in cytokine gene regulatory regions that affect transcriptional accessibility also affect their DNase I sensitivity and can be detected in assays using this enzyme [41,42]. DNase I cleaves DNA at the minor groove of the double helix in regions that are accessible for transcription [43]. Alternatively, DNA regions that are strongly associated with histone proteins, and are therefore transcriptionally inaccessible, are protected from DNase I digestion. Hence, analyzing DNA cleavage products following DNase I digestion provides an indirect assessment for transcriptional accessibility in a DNA region of interest. T-bet and GATA3 expression in undifferentiated helper T cells causes chromatin modifications that can be detected by DNase I hypersensitivity in the IFN-γ and IL-4 genes, respectively indicating transcriptional accessibility [44–46]. This DNase I hypersensitivity and the associated transcriptional activation of the IFN-γ and IL-4 genes is largely due to T-bet and GATA3 induced changes in both DNA methylation and histone acetylation within the IFN-γ and IL-4 loci. H3 and H4 histone acetylation are substantially increased in the IFN-γ and IL-4 loci upon differentiation into Th1 and Th2 cells, respectively [47]. These changes in histone acetylation are lineage and locus specific, and are maintained by T-bet and GATA3 in a STAT signaling-dependent manner [47,48]. In contrast, histones in the IFN-γ and IL-4 loci are hypoacetylated in naïve undifferentiated T cells [47,48]. Histone acetylation provides locus accessibility for transcription of IFN-γ and IL-4 in Th1 and Th2 cells, respectively. The effect of T-bet in inducing IFN-γ transcription in Th1 cells is in part due to increased expression of the homeobox transcription factor Hlx [49]. Hlx expression is induced in Th1 cells by T-bet, and the interaction between T-bet and Hlx induces maximal IFN-γ production [49]. The ability of retrovirally introduced Hlx to promote IFN-γ transcription during early stages of CD4 + T-cell differentiation but not during later differentiation stages suggests that a permissive epigenetic state of the IFN-γ gene is necessary for Hlx-induced IFN-γ transcription [50]. This emphasizes the role of T-bet in inducing chromatin accessibility, as coexpression of T-bet in later stages of helper T cell differentiation allows Hlx to promote expression of IFN-γ [50].

DNA methylation has been shown to play an important role in regulation of IFN-γ expression. Methylation of three CG sites in the 5′ flanking region of IFN-γ gene correlates with transcriptional silencing [51]. In naïve CD4 + T cells, which have a low capacity to express IFN-γ, IFN-γ is heavily methylated in all 3 CG sites. In contrast, the IFN-γ gene is hypomethylated in T-cell lineages having a high capacity to produce IFN-γ [51]. Upon differentiation into Th1 cells, the promoter of the IFN-γ gene becomes hypomethylated, while the IL-4 gene is silenced by DNA methylation [49,52]. The development of Th2 cells, on the other hand, is associated with hypomethylation in the 5′ region of the IL-4 locus and heavy methylation of the IFN-γ locus [53–55] (Figure 3). Genes encoding Th2 cytokines (IL-4, IL-5 and IL-13) are clustered in a locus that also contains the RAD50 gene, which is involved in DNA repair. The frequently co-regulated expression of IL4, IL5 and IL13 is explained by the "loop" configuration of this locus, which brings a locus control region (LCR) in the RAD50 gene near the promoter regions of IL-4, IL-5 and IL-13 genes [56,57]. The LCR contains several Th2 specific DNase I hypersensitivity sites [42]. One of these, hypersensitive site 7 (RHS7), undergoes rapid and complete Th2-specific demethylation within two days of TCR stimulation [42]. Interestingly, RHS7 deficient mice show an impairment in the Th2 response as indicated by a significant reduction of IL4, IL5, and IL13 expression following peptide immunization [58].

Th17 differentiation

A more recently recognized distinct helper T-cell subset is the Th17 lineage. Th17, once thought to arise from a common Th1 precursor, differentiates independently from naïve CD4 + T cells in the presence of IL-6 and TGF-β [59]. IL-23 has a role in maintaining Th17 survival and expansion, but probably does not contribute to Th17 differentiation as was previously suspected [59,60]. The signature cytokines produced by Th17 cells are IL-17 (IL-17A) and its homolog IL-17F, which have genes located on the same region of chromosome 6p12 in humans. Th17 cells are known to be involved in the pathogenesis of a number of autoimmune diseases and this continues to be an area of active investigation [26,60]. Similar to Th1 and Th2 differentiation, helper T-cell differentiation and polarization into Th17 is accompanied by chromatin remodeling in both the IL-17 and IL-17F genes [61]. H3 acetylation and lysine 4 trimethylation in IL-17 and IL-17F promoter regions are associated with Th17 lineage development [61]. Histone acetylation in IL-17 and IL-17F promoter regions in the presence of both IL-6 and TGF-β functionally correlates with the activation of the IL-17 and IL-17F genes [61]. This suggests that

both IL-6 and TGF-β promote chromatin remodeling and therefore transcriptional accessibility of the IL-17 and IL-17F genes in a cytokine environment permissive for Th17 development.

Role of DNA methylation in IL-2 expression

Interleukin 2 (IL-2) is an important cytokine that maintains survival and proliferation of activated T cells. Upon activation, T cells express both IL-2 as well as the high affinity IL2Rα which is also known as CD25. In naïve T cells, the IL-2 gene is silenced but is expressed upon T-cell activation. Indeed, within the region from −2.2 Kb to +98 bp relative the IL-2 transcription start site, all 15 CG sites are methylated in naïve T cells [62]. However, after 7 h of T-cell activation *in vitro*, a small region in this promoter/enhancer region of IL-2 gene, containing 5 CG sites, is demethylated [62]. To determine the functional significance of this methylation–demethylation on gene expression, transfection of pre-activated T cells with a luciferase reporter gene driven by an IL-2 promoter was performed. Methylation of this region inhibited expression of the reporter gene; however, when methylation was inhibited by inducing point mutations in the 5 CG sites that are demethylated in stimulated T cells, a significant increase of luciferase activity was detected [62]. This implies that demethylation of the IL-2 promoter induces IL-2 expression in activated T cells. *In vivo* experiments showed that demethylation in the IL-2 promoter occurs as early as 20 min after stimulation, and is essentially competed before DNA replication [62,63]. The speed with which DNA is demethylated in the IL-2 gene upon T-cell activation and the absence of any link to DNA replication implies the existence of DNA demethylase enzyme(s) [63]. Furthermore, demethylation in the IL-2 promoter/enhancer in activated CD4 + T cells appears to be a downstream effect of CD28 co-stimulation, which is also associated with marked histone acetylation and chromatin remodeling in the IL-2 promoter region [64].

DNA methylation and regulatory T-cell function

Naturally occurring regulatory T cells (Treg) are CD4 + T cells constitutively expressing CD25. The development of autoimmunity in thymectomized mice, rescued by CD4 + cell transfer, was an important clue with respect to the function of a CD4 + T-cell subset in suppressing and controlling immune responses (reviewed in Ref. [65]). Expression of the fork-head transcription factor FOXP3 is known to be critical for the development and regulatory functions of Tregs [66]. Indeed, deletion or mutation of FOXP3 in mice or humans, respectively, is associated with development of autoimmunity [66,67]. Further, ectopic expression of FOXP3 in primary T cells leads to the development of a T-cell subset with suppressive functions [66]. The expression of FOXP3 and thus the generation of Tregs can be induced in the presence of TGF-β [68]. The regulation of FOXP3 expression is not completely understood; however, recent findings indicate a pivotal role for DNA methylation. Regulatory T cells (CD4 + CD25 + FOXP3 +) isolated from secondary lymphoid organs of male BALB/c mice showed complete demethylation in an evolutionarily conserved transcription element of the FOXP3 promoter [69]. In contrast, naïve CD4 + T cells (CD4 + CD25 −) showed almost complete methylation of the same CG sites that corresponded with a lack of FOXP3 expression in these cells [69]. Furthermore, chromatin immunoprecipitation assays revealed increased histone acetylation and H3 lysine 4 trimethylation within the conserved region in the FOXP3 promoter of CD4 + CD25 + Tregs [69]. This suggests that DNA demethylation in this region causes increased chromatin accessibility at the FOXP3 locus. Indeed, stimulated CD4 + CD25 − T cells treated with the DNA methylation inhibitor 5-azacytidine exhibit increased FOXP3 expression [70]. The same region of FOXP3 is demethylated in CD25 + but not CD25 − CD4 + single positive thymocytes from male BALB/c mice. In double negative or double positive thymocytes, where FOXP3 is not expressed, CG sites within that region were completely methylated [69]. Another transcriptionally relevant CG-rich region was identified in the first intron of FOXP3 [70]. FOXP3 expression is inversely correlated with methylation of this region, which is demethylated in CD4 + CD25 + cells similar to the CG-rich region in the FOXP3 promoter. TGF-β, which induces Treg differentiation, decreases DNA methylation in both the promoter and intronic CG-rich regions corresponding with increased FOXP3 expression [70]. These findings imply an important role for epigenetic regulation in the differentiation and function of Tregs.

Conclusion

Epigenetic mechanisms are clearly involved in regulating normal immune responses. This is especially clear in the differentiation of helper T cells into various subsets having specific well defined functions as determined by their cytokine production. By mediating heritable changes in chromatin accessibility that affect gene expression, epigenetic mechanisms promote the continuation of the differentiated effector T-cell response throughout out cell proliferation occurring during clonal T-cell expansion. As our understanding of epigenetic regulation of the immune response evolves, we are likely to uncover important pathological consequences related to epigenetic defects. Indeed, the role for abnormal T-cell DNA

methylation is well established in autoimmunity, in particularly systemic lupus erythematosus (reviewed in Ref. [71]). More work is clearly needed to define the full role of epigenetic modulation in immune responses during both health and disease.

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