Helper T-cell differentiation and plasticity: insights from epigenetics

Kiyoshi Hirahara,* Golnaz Vahedi,* Kamran Ghoreschi, Xiang-Ping Yang, Shingo Nakayamada, Yuka Kanno, John J. O’Shea and Arian Laurence

Department of Lymphocyte Cell Biology Section, Molecular Immunology and Inflammation Branch, National Institutes of Arthritis, and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD, USA

doi:10.1111/j.1365-2567.2011.03483.x
Received 8 June 2011; revised 1 July 2011; accepted 5 July 2011.
*Kiyoshi Hirahara and Golnaz Vahedi contributed equally to the writing of this article.
Correspondence: K. Hirahara, National Institutes of Health, 10 Center Dr, Bldg10, Room 13C103 Bethesda, MD 20892-1930, USA. Email: hiraharak@mail.nih.gov
Senior author: Arian Laurence, email: laurencea@mail.nih.gov

Summary

CD4+ T cells have critical roles in orchestrating immune responses to diverse microbial pathogens. This is accomplished through the differentiation of CD4+ T helper cells to specialized subsets in response to microbial pathogens, which evoke a distinct cytokine milieu. Signal transducer and activator of transcription family transcription factors sense these cytokines and they in turn regulate expression of lineage-defining master regulators that programme selective gene expression, resulting in distinctive phenotypes. However, phenotype and restricted gene expression are determined not only by the action of transcription factors; chromatin accessibility is required for these factors to exert their effect. Technical advances have greatly expanded our understanding of transcription factor action and dynamic changes in the epigenome that accompany cellular differentiation. In this review, we will discuss recent progress in the understanding of how cytokines influence gene expression and epigenetic modifications, and the impact of these findings on our views of helper cell lineage commitment and plasticity.

Keywords: epigenetics; histone modification; signal transducer and activator of transcription; T-cell differentiation; T-cell plasticity

Introduction

The critical role of CD4+ T cells in host defence and immunoregulation is well-appreciated. Initially, the fates of CD4+ T helper (Th) cells were thought to be limited to Th1 and Th2 cells. Th1 cells, which express the transcription factor T-bet (encoded by the Tbx21 gene) and produce interferon-γ (IFN-γ) exclusively, protect the host against intracellular infections including viruses and Toxoplasma. Th2 cells, which express GATA-3 and secrete interleukin-4 (IL-4), IL-5 and IL-13, mediate host defence against helminths. More recently, cells that selectively produce IL-17 have been proposed to represent a distinct Th-cell lineage, Th17 cells. Th17 cells can also produce other cytokines including IL-9, IL-10, IL-21, IL-22 and, in humans, IL-26; however, these cytokines can also be produced by other subsets. Th17 cells express a splice variant of the retinoid orphan receptor-γt, designated RORγt. Th17 cells contribute to host defence against extracellular bacteria and fungi. Other putative lineages have also been identified including Th9 and Th22, which produce their namesake cytokines IL-9 and IL-22, respectively.

Follicular helper T (Tfh) cells have recently been proposed as yet another lineage, with specialized function in helping B cells make antibody responses. As with other helper cell lineages, extrinsic cytokine cues, namely IL-6 and IL-21, acting via signal transducer and activator of transcription 3 (STAT3), have been proposed to be the principal drivers of Tfh-cell differentiation. These factors induce expression of Bcl6 and current models posit that Tfh cells arise from naive T cells and through acquisition of this transcription factor, which has come to be viewed as the master regulator of Tfh cells.
and Bcl6 are antagonistic and reciprocal regulators of Th differentiation, and it has been argued that balance of expression of these factors is important for Th versus effector cell differentiation. 25,27 These findings have been invoked to support the lineage sovereignty of Th cells.

Regulatory T (Treg) cells are another CD4+ lineage with essential immunosuppressive functions and expression of the master regulator transcription factor FoxP3. Thymically derived Treg cells are referred to as natural Treg (nTreg) cells, whereas Treg cells that arise from naive CD4+ T cells in the periphery are denoted induced Treg (iTreg) cells. 28–30

In addition to their role in host defence, different T-cell subsets are also associated with immune-mediated disease. For example, Th1 and Th17 cell responses have been associated with a number of autoimmune diseases, 31–36 whereas Th2 cytokines are associated with atopic disease. 37–40 Absence of regulatory T cells because of mutations of FOXP3 results in severe, systemic autoimmunity. 41–43

All of the subsets can be generated in vitro by activating naive CD4+ cells in specific cocktails of cytokines. That is, T-cell receptor engagement in the presence of IL-12 and IFN-γ and the absence of IL-4 yields Th1 cells. 44 In contrast, the presence of IL-4 and absence of IFN-γ results in Th2 polarization. 45 The remaining two lineages can be generated in the presence of transforming growth factor-β (TGF-β) with the combination of TGF-β and IL-2 producing iTreg cells and the combination of TGF-β and IL-6 producing Th17 cells, 46,47 although Th17 cells can also be made in the absence of TGF-β signalling. 48

Many of the aforementioned cytokines that promote helper cell specification bind to receptors that are members of the Type I/II cytokine receptor superfamily and signal by the JAK/STAT pathway. There are seven STAT proteins (STAT1-5a, 5b and 6). Mice that are deficient in any of these STAT proteins function as sensors of the cytokine environment. 49,50

Hence, characteristics of various Th subsets include distinct immunological functions, the expression of a unique repertoire of cytokines, and the expression of a master regulator of transcription factors induced by exogenous factors. These subsets have been viewed as ‘lineages’ in that the phenotypes are often stable and hereditable; however, there are also more examples of flexibility. In this review, we describe a few of the many examples of Th phenotypic plasticity, the epigenetic changes that occur in CD4+ T-cell differentiation and how these epigenetic modifications contribute to CD4+ T-cell heritability and plasticity. Moreover, we will briefly review how STAT proteins function as sensors of the cytokine environment and promote the acquisition of epigenetic changes.

**Helper cell lineage commitment versus plasticity**

The standard model of Th1, Th2 and Th17 differentiation implies that these subsets behave like terminally differentiated cells with a heritable expression of specific cytokines and transcription factors. To some extent this is correct – generally speaking, Th1-polarized cells make IFN-γ and do not make IL-4 or IL-17. Similarly, nTreg cells behave as a reasonably stable lineage in vivo. 59 However, other findings indicate more flexibility than envisioned and recent findings provide mechanisms for flexibility in expression of key transcription factors.

**Flexibility of Th2 cells**

Although the established Th1/Th2 dogma would imply that these subsets conform to a lineage commitment model, recent data have provided some rather dramatic surprises that challenge this view. Hegazy et al. 60 showed that committed GATA-3-Th2 cells could be reprogrammed to adopt GATA-3+T-bet+ and IL-4+IFN-γ− phenotype by lymphocytic choriomeningitis virus (LCMV) infection, and these GATA-3 T-bet double-positive cells were critical to LCMV exclusion.

**Intrinsic instability of Th17 cells**

Initially, IL-17-secreting Th cells were suggested to represent a new lineage, as the cells did not make other lineage-defining cytokines like IFN-γ and IL-4. 7 Like Th1 and Th2 cells, Th17 cells could be induced in vitro from naive T cells and required a unique combination of cytokines (TGF-β and IL-6) 61 and the intracellular signalling molecule, STAT3. 57,62,63 Factors distinct from those required for classical Th1/Th2 polarization. Th17 cells, like their Th1/2 counterparts expressed unique master regulator transcription factors (RORγt, RORα) 64. Finally, cytokines that drove Th1 and Th2 lineage commitment were potent inhibitors of Th17 development. 7 Interestingly, IL-2 shows the reciprocal effects on Treg and Th17 differentiation; IL-2 promotes Treg-cell differentiation, while IL-2 negatively regulates Th17 differentiation; both of these effects being dependent upon STAT5. 58,65–67

Once polarized, relatively rapid extinction of IL-17 expression occurred with acquisition of IFN-γ expression. 68 This was initially suspected in models of experimental autoimmune uveitis where transferred antigen-specific in-vitro-generated Th17 cells subsequently became IFN-γ producers. 69 Similarly, tumour immunity mediated by Th17 cells required the expression of IFN-γ. 70 These initial results were confirmed in vitro using T cells from IL-17F reporter animals, where pure populations of Th17-secreting cells could be isolated on the basis of expressing fluorescent proteins and were subsequently found to convert to IFN-γ-producing cells. 68
More recently, Stockinger and colleagues, using a fate mapping reporter strategy, demonstrated that this conversion occurs physiologically. As indicated, T-bet is a key Th1 transcription factor, and recent work indicates that T-bet suppresses Th17 differentiation by preventing Runx1-dependent transactivation of Rorc. We and others have identified the rapid appearance of RORc+ T-bet double-positive cells. In a murine experimental autoimmune encephalomyelitis model, RORc+ T-bet double-positive cells showed greater pathogenic potential compared with RORc+ T-bet− cells.

In contrast to the multiple lines of evidence pointing to conversion of Th17 to Th1 cells, until recently there was relatively little evidence of overlap between Th17 and Th2 cells; although, the two lineages are both dependent on the transcription factor, Interferon regulatory factor 4 (IRF4). Recently, a subset of GATA3 RORc+ double-positive cells were identified and these cells were reported to play a role in the development of experimental asthma. In this regard, it is of interest that severity of asthma correlates with serum IL-17 concentration.

**Tfh cells – lineage or state?**

As the newest 'lineage' it is perhaps not surprising that there is considerable controversy surrounding the origin and fate of Tfh. Activated CD4+ T cells are not devoid of Bcl6 and the signature Th1 cytokine, IL-21, is not exclusively produced by Tfh cells. An added complication is that Tfh cells have the capacity to produce cytokines characteristic of Th1, Th2 and Th17. Furthermore, helminths infection results in GATA3 Bcl6 double-positive Tfh cells. Moreover, FoxP3+ T cells to Peyer’s patches can convert to Tfh cells. It remains an open question whether Tfh cells represent a distinct lineage parallel to other subsets of helper T cells, a temporary 'state' of differentiation or a phenotype that can be superimposed upon Th1, Th2, Th17 or Treg cells.

**Stability of Treg cells – ongoing controversy**

Treg cells are an essential subset, whose function is to inhibit immune responses. They are characterized by the expression of the inhibitory transcription factor, FoxP3, mutation of which results in a rapidly fatal inflammatory disease in both mice (Scurfy) and humans (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; IPEX). Conversely, administration of Treg cells has been proposed as cellular therapy in transplantation and autoimmune disease. This notion though is predicated on the assumption that Treg cells injected into patients will maintain their immunosuppressive phenotype and not become effector cells. However, using a fate mapping approach, it was argued that loss of FoxP3 expression was not infrequent and that Treg cells had the capacity to become IL-17-secreting cells that could induce tissue destruction. Furthermore, in response to IL-12 in vitro or in the setting of Toxoplasma infection or colitis, Treg cells can produce IFN-γ. The loss of suppressive function is not necessarily seen upon acquisition of IFN-γ production. Interestingly, a unique Treg subset exists at sites of inflammation, which expresses both FoxP3 and T-bet and inhibits Th1 inflammation. Similarly, mice that lack the Th2 and Th17-associated transcription factor IRF4 in Treg cells develop Th2-associated inflammation. Despite these findings, other work has argued that nTreg cells maintain their phenotype in the setting of Listeria infection.

In contrast to mouse cells, FoxP3 is a less reliable marker of suppressive activity, being induced in activated human T cells. Both of CD45RA+ FoxP3low and CD45RA− FoxP3high cells have suppressive activity and CD45RA− FoxP3low cells have the capacity to convert into CD45RA− FoxP3high cells.

Collectively, the data indicate that although helper cells have features of phenotypic stability, it is clear that they also have the capacity for considerable plasticity. This then raises the broader question of what factors preserve the phenotype of specialized cells.

**Epigenetics and cellular phenotype**

The issue of flexibility versus stability of phenotype is certainly not a concept that is unique to T cells. On the contrary, it has long been appreciated by developmental biologists that once differentiated, cells maintain their phenotype, even in the absence of exogenous signals. That is, terminally differentiated cells autonomously maintain their distinctive features even through subsequent mitotic division. Though the term ‘epigenetics’ was coined by Waddington before the era of modern molecular biology, it has come to denote hereditable changes in phenotype or gene expression without changes in DNA sequence. Increasingly, epigenetics has come to encompass various aspects of nucleosome biology and DNA methylation, which help to explain selective gene expression. Indeed, it is now well accepted that cellular phenotype is the result of the action of transcription factors regulating gene expression, but that the accessibility of genes to activation is also highly regulated through epigenetic modifications.

The basic structural unit of chromatin is the nucleosome, which consists of two copies of histones H2A, H2B, H3 and H4. Each nucleosome is encircled by 146 base pairs of DNA and interconnected by linker DNA. Selective positioning of nucleosomes by ATP-dependent chromatin remodelling complexes is one important aspect of genomic regulation. DNA methylation is another modality that limits the accessibility of DNA to cellular machinery, thereby affecting the transcriptional potential of the underlying DNA sequence.
Additionally, the N-terminal tails of histone proteins can be covalently modified through acetylation, methylation and phosphorylation. Histone modifications are associated with the regulation of gene expression by condensing or relaxing the chromatin structure to repress or activate transcription, respectively. For example, trimethylation of H3K4 (H3K4me3) is associated with gene activation. In contrast, polycomb complexes catalyse trimethylation of H3K27 (H3K27me3), which serves to repress gene expression. Initially discovered as regulators of the Drosophila homeotic genes, polycomb-dependent H3K27me3 is associated with a wide range of targets in mammals.

As we learn more about the biochemistry of histone modifications and the enzymes responsible for the plethora of covalent modifications, it is recognized that the regulation of chromatin is a highly dynamic process. Indeed, some modifications can occur rapidly in response to exogenous signals and hence the regulation of nucleosome biology can be viewed as an extension of signal transduction. Yet, it remains unclear what signals a cell must receive and send to organize the epigenome with respect to its roles in developmental biology, cell differentiation, or stem cell renewal. It is worth emphasizing that epigenetic modifications also have the ability to persist in the absence of continued exogenous signals and even the transcription factors that initially induced them. This allows a cell to ‘remember’ its distinctive transcriptional profile and, by extension, its cellular identity.

Recent studies have demonstrated that epigenetic modifications are also important factors for the regulation of Th-cell differentiation. DNase hypersensitive sites, DNA methylation and chromatin modifications at candidate loci in Th cells such as IFNG, IL-4 and Foxp3 have been analysed. For instance, DNase I hypersensitive sites in the Ifng locus are induced upon Th1, but not Th2, differentiation. Similarly, a conserved CpG-rich region denoted as a Treg-specific demethylated region resides upstream of the Foxp3 promoter; this region is demethylated in nTreg cells and is associated with stable expression of FoxP3. In contrast, there is regional methylation of this region in iTreg cells, consistent with less stability in expression.

Alternatively, the potential importance of epigenetic modifications in Th-cell differentiation has been inferred through the use of different gene knockout mice. For instance, haplo-insufficiency of the H3K4 methyltransferase MLL (MLL+/−) resulted in defective maintenance but not initial induction of Il4, Il5, Il13 and Gata3 expression in Th2 cells. EZH2, a major H3K27 methyltransferase, binds to the Il4/I13 locus and has been reported to be responsible for the suppression of the Il4/I13 locus in Th1 cells. However, a complicating factor is that Ezh2 also plays an essential role in actin polymerization-dependent processes such as antigen receptor signalling in T cells. Chromatin remodelling complexes, which contain Brahma-related gene 1 (BRG1), displace nucleosomes and remodel chromatin at the Ifng promoter region in a Th1-specific manner and knockdown of Brg1 results in decreased IFN-γ production. Originally thought to principally mediate sister chromatid cohesion during mitosis, cohesin and CCCTC-binding factor (CTCF) also occupy a subset of DNase I hypersensitive sites in the Ifng locus. Knocking down the expression of RAD21, one component of the cohesin complex, reduces IFNG transcript levels.

In recent years, there has been a remarkable increase in our ability to study the structure of chromosomes, particularly through the development of chromosome conformation capture (3C) technology. 3C employs restriction enzymes to fragment DNA and regular PCR to detect ligation products. Studying the chromatin configurations revealed that chromatin is folded into loops bringing distal regulatory elements into intimate contact with the genes that they regulate.

One of the first systems for which the intrachromosomal and interchromosomal associations have been reported is the differentiation of naive CD4+ Th cells into Th1 and Th2 subsets. Using the 3C technique, it was found that in CD4+ cells, but not natural killer cells, B cells and fibroblasts, the promoters for the genes encoding Th2 cytokines are located in close spatial proximity with the Th2 locus control region. A similar study revealed that depending on the expression profile of the cell, the Ifng locus adopts a secondary structure that brings the enhancer elements and Ifng promoter into close proximity. It was also shown that deletion of Rad50 hypersensitive site 7 (RHS7) did not affect the expression of the Rad50 gene, but it reduced long-range intrachromosomal interactions between the Th2 locus control region and promoters of the Th2 cytokine genes. In addition to these intrachromosomal interactions, the interchromosomal interactions between the promoter region of the IFN-gamma gene on chromosome 10 and the regulatory regions of the Th2 cytokine locus on chromosome 11 was also described.

Recent studies indicate that microRNA (miRNA) molecules may also have important regulatory roles in transcription. The miRNAs are small RNAs that bind to target messenger RNAs (mRNAs) and lead to inhibition of translation or mRNA degradation. A central role for miRNAs in maintaining the stability of differentiated Treg cell function in vivo and homeostasis of the adaptive immune system has also been shown. Moreover, it was shown that lymphocyte-specific miRNAs are either tightly controlled by polycomb group-mediated H3K27me3 or maintained in a semi-activated epigenetic state before full expression. Collectively, these data argue that epigenetic changes influence fate determination of helper T cells.
Technical advances: next generation sequencing, ChIP-seq

Until recently, analysis of epigenetic changes has necessarily aimed at very limited regions of the genome, focusing on pre-selected elements (promoters or recognized enhancers). The problem with such an approach is the tacit assumption that we know the key players. Obviously though, this may or may not be the case. With the advent of next-generation sequencing methods, an unbiased genome-wide view of epigenetics modifications has become a reality.

This technique involves cross-linking of DNA/protein complexes and immunoprecipitation using antibodies directed at transcription factors or other DNA-binding proteins. Antibodies directed at the many specific histone modifications can also be employed. After reversing the cross-links, the isolated DNA can be sequenced using next-generation sequencing technology to produce digital maps of enrichment. This technique, referred to as chromatin immunoprecipitation and massive parallel sequencing (ChIP-seq) permits genome-wide profiling of DNA-binding proteins, DNA methylation, histone modifications or nucleosome positioning. Comprehensive mapping of 13 transcription factors in embryonic stem cells has now been obtained. Another example of using deep sequencing is the genome-wide mapping of histone acetyltransferases (HATs) and deacetylases (HDACs) binding on chromatin where it was shown that both are found at active genes with acetylated histones.

Several methods have been developed to profile DNA methylation. Such regions can be identified based on the enrichment of methylated genomic DNA using an antibody specific for methylated cytosines. This method can provide relatively inexpensive and comprehensive data but the resolution is limited. Alternatively, sequencing of bisulphite-converted DNA, which provides base-resolution but at a higher cost, can also be employed. Complete DNA methylomes for several organisms have now been obtained, including a genome-wide map of methylated DNA in a mammalian genome from both human embryonic stem cells and fetal fibroblasts. High-throughput sequencing of 5-hydroxymethylcytosine, a modified base present at low levels in diverse cell types, from mouse embryonic stem cells, has also been completed. These data should clarify the evolutionary significance of this epigenetic mark and its distribution in key genomic elements.

The development of high-throughput DNA sequencing methods can also be used for both mapping and quantifying transcriptomes. This method, termed RNA-Seq (RNA sequencing), has clear advantages over existing approaches and is expected to revolutionize the manner in which eukaryotic transcriptomes are analysed.

In summary, these new approaches permit the shift away from gene-centric paradigms to the genome-wide views of the actions of transcription factors and their impact on the global epigenetic and transcriptional status of cells.

Insights into CD4+ T-cell plasticity from epigenetic analysis

The profiling of 39 different histone marks in human CD4+ T cells has been now been accomplished. These maps have associated particular modifications with gene activation or repression and with various genomic features, including promoters, transcribed regions, enhancers and insulators. In addition, genome-wide H3K4me3 and H3K27me3 mapping of naive, Th1, Th2, Th17, iTreg and nTreg cells have also been obtained. This information provided some surprises but has also helped to explain some of the behaviour of helper T cells. Consistent with the standard ‘lineage commitment’ model of helper-cell differentiation, proximal promoters of cytokine genes show permissive (H3K4me3) marks on lineage-defining cytokines in the respective lineages (e.g. Ifng in Th1 cells), which are accompanied by repressive (H3K27me3) marks in other subsets (Fig. 1). A surprise though, was that the histone methylation patterns of genes encoding key transcriptional factors that drive lineage specification exhibit both repressive and accessible marks in subsets of cells in which the master regulators were not expressed. For example, the Tbx21 promoter is associated with H3K4me3 marks in Th1 cells. While H3K27me3 marks are present in Th2 and Th17 cells, H3K4me3 marks are also present even though the transcription factor is not expressed (Fig. 2). Such regions, marked by both chromatin modifications, are termed bivalent domains and have been seen in genes poised for expression in stem cells. This then can help explain how T-bet might be induced in Treg, Th17 and even Th2 cells. In this context, it is
perhaps not surprising that Th17 cells generated in vitro are intrinsically unstable. Indeed this instability is correlated with the rapid changes of the epigenetic modifications when Th17 cells are stimulated in the presence of IL-12. In contrast, isolated memory Th17 cells appear to be more stable and although it was not shown, their stability was hypothesized to relate to the epigenetic landscape of memory cells. The Gata3 locus was also noted to exhibit bivalent domains and so helps to explain how a novel subset of Th2 memory/effector cells, which express GATA3 and RORγt and produce Th17 and Th2 cytokines, might arise. Similarly, Foxp3 was associated with strong H3K4me3 marks in Treg cells and H3K27me3 was detected in Th1 and Th2 cells as expected. However, no H3K27me3 repressive marks were present in the Foxp3 locus in Th17 cells, consistent with the evidence that Foxp3 can be expressed in Th17 cells.

STATs and the control of the epigenetic landscape of differentiating helper T cells

Since the global epigenetic landscape of the different helper cell subsets is distinct, the question immediately arises, what factor(s) are responsible for the unique epigenetic profiles? As STAT family transcription factors are critical for sensing the cytokine milieu and promoting Th-cell differentiation, it seemed logical to consider that they might contribute to the distinctive modifications seen in helper cell subsets. However, the extent to which STATs are drivers or followers of epigenetic modifications in T cells was by no means clear, and in fact, initial studies analysing IFN-γ signalling and STAT1 implied that epigenetic changes largely preceded STAT1 binding. The first issue to tackle was to define STAT target genes in helper cell subsets. Though it has widely been accepted that STATs are critical mediators of gene transcription, transcriptional profiling did not distinguish between
directly and indirectly regulated genes. However, ChIP-Seq technology has the capacity to map DNA-protein binding sites genome-wide. In addition, through the use of gene-targeted mice and microarray analysis, one can relate transcription factor binding with factor-dependent gene regulation. In addition, STAT-dependent global epigenetic modifications can also be ascertained. Using ChIP-seq, STAT4 and STAT6 were found to have around 12,000 and 13,000 binding sites in Th1 and Th2 condition, respectively. Comparative epigenetic analysis of wild-type versus STAT4-deficient Th1 cells provided evidence that of the approximately 4000 genes bound by STAT4, nearly 1000 had STAT4-dependent alterations in epigenetic modifications. And of these 1000 genes, 200 had highly STAT4-dependent gene expression, as determined by microarray analysis of wild-type versus STAT4-deficient cells. Similarly, of around 4000 genes bound by STAT6, the epigenetic modifications in 970 of them were highly dependent on STAT6. Many of the expected target genes fell into this category. For instance, Ifng, Thx21, Furin and Il18r in Th1 cells and Il4, Gata3, Il24 and Zbtb32 in Th2 cells. Using the same approach, we mapped STAT3 binding sites in Th17 cells. We found that STAT3 bound to more than 3000 genes and among its targets were many genes implicated in Th17-cell differentiation including Il17a, Il17f, Il21 and Il6ra. Interestingly, STAT3 binds to multiple sites in the Il17 locus, the most prominent of which are intergenic regions that coincide with conserved non-coding sequences.

There appear to be subsets of genes that are highly dependent on STATs for promoting both gene expression and the local epigenetic environment. Interestingly though, there were subtle differences in how STAT4 and STAT6 affected the epigenetic landscape. Specifically, in a significant portion of genes STAT4 served to enhance H3K4me3 marks. In contrast, the global effect of STAT6 was to predominantly regulate H3K27me3 modifications.

Of particular interest is a subset of genes that are bound by STAT4 in Th1 cells but by STAT6 in Th2 cells, for which the two STATs have opposing effects on local epigenetic patterns. A notable example is the Il18rap locus (Fig. 3). Whereas one STAT (in this case STAT4) promotes permissive marks in Th1, the other STAT (STAT6) is critical for establishing repressive marks.

Figure 3. Opposing effects of signal transducer and activator of transcription 4 (STAT4) and STAT6 on epigenetic modifications in the Il18rap locus. Both STAT4 and STAT6 bind to the promoter region of the Il18rap locus in T helper type 1 (Th1) and Th2 cells, respectively. In this locus, STAT4 is required for permissive (H3K4me3) marks in Th1 (upper panel), as these marks are absent in Stat4-deficient Th1 cells. On the other hand, STAT6 is critical for establishing repressive (H3K27me3) marks on the same locus in Th2 cells (lower panel), as evidenced by the absence of these marks in Stat6-deficient Th2 cells. This is an illustrative example of the ways that two STATs may have opposing effects on local epigenetic patterns.
on the same locus in Th2 cells. This divergent action of STAT4 and STAT6 on the same gene provides a mechanism for preserving gene expression in one lineage while ensuring repression in the opposing lineage.

These new data revealed yet another mechanism of opposing regulation. As discussed, IL-2 acting through STAT5 inhibits IL-17 production. Using genome-wide ChIP-seq analysis, we found that STAT3 and STAT5 bound the same binding sites in the Il17a-Ill17f locus in CD4⁺ T cells. These data suggested the possibility that STAT5 could directly compete with STAT3 for binding to enhancer elements within the Il17a-Ill17f genetic locus. In fact, that was the case; IL-2-dependent STAT3 binding by IL-2 was associated with a reduction of STAT3 binding at multiple sites throughout the Il17a-Ill17f locus in Th17 cells (Fig. 4). STAT5 recruitment by IL-2 is associated with a reduction in positive epigenetic modifications at these sites, including histone H3 acetylation and H3K4me3 but did not enhance H3K27me3 accumulation (Fig. 4). These data suggest a novel mechanism whereby STAT proteins can repress gene expression not just by promoting the accumulation of repressive marks, but also by direct competition with one another to regulate gene transcription.

Conclusions and prospects for the future

In the last few years, there has been an avalanche of new information pertaining to novel Th-cell ‘lineages’. These discoveries have enhanced our understanding of how a key player, the CD4⁺ T cell, can attain different fates and thereby orchestrate the magnitude and character of immune responses. However, the flexibility versus stability of these phenotypes and the extent to which these fates represent specification and commitment or meta-stable states remain to be determined.

It is well-appreciated that specific cellular phenotypes are associated with distinctive transcriptional profiles and that gene expression is controlled by both trans-acting factors and cis-elements, but in addition a deeper understanding of epigenetics is rapidly emerging. With increasing evidence indicating a close relationship between the gene transcription, transcription factor binding and the state of chromatin in CD4⁺ T cells, we will hopefully gain insight into how extrinsic cytokines provide signals that induce expression of key transcription factors and how these factors work in concert to change and maintain the helper cell phenotype.

Driven by technological advances, new methods have allowed us to investigate the actions of transcription factors and the ways they modify the epigenome on a genome-wide scale. In addition to better understanding how histone modifications change during the course of helper cell differentiation, a genome-wide view of DNA methylation in CD4⁺ T cells also remains to be obtained. How histone modifications relate to the gain and loss of DNA methylation eventually need to be sorted out, with emphasis on defining the functional significance of these changes.

In addition to promoters, distal enhancer elements are recognized as a major means of controlling tissue-specific gene expression and functioning in a cell-type-specific manner. Importantly, a chromatin signature for enhancers is now recognized and enhancers have been found to be preferentially occupied by the co-activator, p300. Relatively few enhancer elements have been defined in helper T cells; however, with ChIP-seq technology, it will be possible to comprehensively map the dynamic enhancer landscape of differentiating CD4⁺ T cells and begin to define factors responsible for the organization of these elements.
Recent studies have shown that various types of RNA also play an important regulatory role in transcription and epigenetic modifications. In addition to miRNAs, long non-coding RNAs (lncRNAs) influence the expression of neighbouring protein-coding genes in multiple cell lines. Such lncRNAs can serve as a scaffold for the assembly of transcription factors or other chromatin remodelling enzymes at the promoter. In the future, it will be important to identify and characterize RNAs that play fundamental roles in the differentiation of Th cells.

The field of chromosome conformation capture is now capable of mapping the three-dimensional organization of chromosomes at a resolution of several kilobase pairs and the scale of the complete genome. Future chromatin interaction mapping of CD4+ T cells and its distinct subsets remains to be charted. Such data could provide insights into dynamic properties of chromatin in Th cells and reveal whether the chromatin has a distinct structure in stable Th subsets.

In summary, an assorted array of techniques is now available that permits genome-wide views of transcription factor action, epigenetic regulation and gene expression. What remains unclear is how the cellular microenvironment governs the changing of the Th-cell epigenome and resultant regulation of gene expression. Over the next few years, we will rapidly obtain a far more sophisticated and comprehensive view of Th-cell differentiation and a better understanding of what are the drivers. Ideally, this avalanche of information will provide increasingly nuanced views of lineage stability and plasticity and how this relates to the transition of naïve cells to specialized effector/memory cells.

Acknowledgements

We would like to acknowledge the assistance of Gustavo Gutierrez-Cruz and the NIAMS Solexa Sequencing Core, and Dr. Hongwei Sun and the NIAMS Biodata Mining Section. This work was supported by NIH/NIAMS Intramural Research Program (IRP).

Disclosures

The authors have no competing financial interests nor conflicts of interest to disclose.

References


Published 2011. This article is a U.S. Government work and is in the public domain in the USA., 134, 235–245
K. Hirahara et al.


