Epigenetic mechanisms regulating T-cell responses

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During the last decade, advances in sequencing technologies allowed production of a wealth of information on epigenetic modifications in T cells. Epigenome maps, in combination with mechanistic studies, have demonstrated that T cells undergo extensive epigenome remodeling in response to signals, which has a strong effect on phenotypic stability and function of lymphocytes. In this review we focus on DNA methylation, histone modifications, and chromatin structure as important epigenetic mechanisms involved in controlling T-cell responses. In particular, we discuss epigenetic processes in light of the development, activation, and differentiation of $CD4^+$ T helper (T_H), regulatory T, and $CD8^+$ T cells. As central aspects of the adaptive immune system, we review mechanisms that ensure molecular memory, stability, plasticity, and exhaustion of T cells. We further discuss the effect of the tissue environment on imprinting T-cell epigenomes with potential implications for immunotherapy. (J Allergy Clin Immunol 2018;142:728-43.)

Key words: *Epigenetics, gene regulation, enhancer, promoter, chromatin accessibility, histone modifications, DNA methylation, transcription factor binding, T-cell development, T-cell function, T-cell exhaustion, regulatory T cells, tissue specificity*

Abbreviations used

ATAC:	Assay for transposase-accessible chromatin
BATF:	Basic leucine zipper TF, ATF-like
CNS:	Conserved noncoding sequence
CTL:	Cytotoxic T lymphocyte
CTLA-4:	Cytotoxic T lymphocyte–associated protein 4
DMR:	Differentially methylated region
DN:	Double-negative
DNMT:	DNA methyltransferase
DP:	Double positive
EZH2:	Enhancer of zeste 2 polycomb repressive complex 2 subunit
FOXP3:	Forkhead box protein P3
GITR:	TNF family member 18
HDAC:	Histone deacetylase
H3K27ac:	Histone H3 lysine 27 acetylation
H3K4me1:	Histone H3 lysine 4 monomethylation
H3K4me3:	Histone H3 lysine 4 trimethylation
Ikzf2:	Ikaros family zing finger 2
IRF4:	Interferon regulatory factor 4
LCR:	Locus control region
PD-1:	Programmed cell death protein 1
ROR:	Retinoic acid–related orphan receptor
STAT:	Signal transducer and activator of transcription
T-bet:	T-box transcription factor 21
TCF1:	Transcription factor 1
Tconv:	Conventional T
TCR:	T-cell receptor
TF:	Transcription factor
T_{FH}:	Follicular helper T
TIL:	Tumor-infiltrating lymphocyte
Treg:	Regulatory T
WGBS:	Whole-genome bisulfite sequencing

T cells are a subtype of white blood cells, which play a major role in the adaptive immune system. They can be classified according to their specific function: MHC class II–restricted CD4-expressing T cells (CD4) can differentiate into specific T_H subtypes. In contrast to this, MHC class I–restricted CD8-expressing T cells (CD8) are classically known for their potential to destroy virus-infected or tumor cells, hence their denomination as cytotoxic T lymphocytes (CTLs). Following development in the thymus from early T-cell precursor cells over CD4 and CD8 double-negative (DN) and double-positive (DP) stages, T cells finally differentiate into CD4 and CD8 single-positive (SP) thymocytes. On recognizing MHC-peptide complexes through their T-cell receptor (TCR), naive $CD4^+$ or $CD8^+$ T cells can differentiate into specific effector populations, which is guided by integrating TCR activation with a variety of other signals provided by immune and nonimmune cells. Importantly, after the initial immune response, a persistent pool of memory T cells maintains the capability to respond rapidly to antigen re-encounter, providing long-lasting protection.

All these phenotypic changes in T cells are accompanied by distinct gene-expression programs that equip T cells with the molecules needed to develop and exert their function, and it is of

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Terms in boldface and italics are detailed in the glossary on page 729.

utmost importance for the integrity of the immune system that T cells establish and maintain those states in a highly controlled manner. This is achieved by diverse noncoding gene-regulatory elements in the genome. Proximal and distal regulatory elements (promoters and enhancers, respectively) can be bound by transcription factors (TFs), which cooperate with other proteins, such as remodeling complexes and epigenetic modifiers, to establish and maintain transcription-permissive or transcription-restrictive chromatin environments in a progressive manner. Accompanied modifications of the DNA and chromatin proteins that contribute to maintaining the cell state without changes in the DNA sequence itself (so-called epigenetic modifications) have been described to play a major role in development, activation, and polarization of lymphocytes.

In this review we focus on DNA methylation, histone modifications, and chromatin structure and discuss the recent global epigenome studies on $CD4^+$ T_H cells, regulatory T (Treg) cells, and CTLs with respect to their development and function under physiologic or pathologic conditions.

EPIGENETIC MECHANISMS AND GENE REGULATION

DNA methylation

In mammals the base cytosine is commonly modified by methylation of its carbon at position 5, predominantly in the context of CpG dinucleotides (mCpG).[1] The methylation mark can be written by *de novo* DNA methyltransferase (DNMT) 3A and DNMT3B with the help of DNMT3L.[2,3] Once established, methylation patterns can be stably transmitted over cell divisions through maintenance DNMT1, and this inheritability designates DNA methylation as a true epigenetic mark.[4,5] Long considered extremely stable, recent discoveries regarding the ***ten-eleven translocation (TET)*** family of proteins established the concept of catalyzed active demethylation (Fig 1, *A*).[6] The effects of DNA methylation on transcriptional activity are complex. At CpG islands (regions of high CpG density), especially if located at promoters, DNA methylation causes repression of transcription, and high methylation levels support processes such as X-chromosome inactivation and imprinting.[1] In contrast, gene bodies of highly expressed genes are heavily methylated, with a possible effect on splicing, whereas a low degree of methylation is found at active gene-regulatory elements that are bound by TFs.[7-9] Hence, using methods of genome-wide DNA-methylation profiling, such as ***whole-genome bisulfite sequencing (WGBS)***, has been proved very useful to delineate cell type–specific aspects of gene regulation, identify cis-acting TFs, and describe cellular ontologies.

DNA methylation can affect gene expression in direct and indirect ways (Fig 1, *B*). For instance, DNA methylation actively blocks the binding of certain TFs, hence restricting regulatory elements from receiving positive transcription signals.[1] Furthermore, it has recently been demonstrated that some TFs can actively recognize methylated DNA, and on binding, such factors can recruit other TFs to remodel a repressed chromatin environment toward an active one.[10] As an indirect mechanism, methylated DNA can be recognized by proteins, including methyl-CpG domain–binding (MDBs) proteins, which are able to recruit other chromatin-modifying enzymes, such as histone deacetylases (HDACs),[11] driving a repressed chromatin environment. Conversely, CXXC-type zinc finger protein 1 recognizes CpG islands and recruits activating histone methyltransferases that create a permissive chromatin environment.[12]

GLOSSARY

CHROMATIN IMMUNOPRECIPITATION (ChIP): An assay used to investigate interaction between proteins and DNA in the cell by evaluating transcription regulation through histone modification or transcription factor–DNA-binding interactions.

DIFFERENTIALLY METHYLATED REGION (DMR): Regions of the genome with different methylation states that are considered functional regions involved in gene transcriptional regulation. Identifying DMRs among different tissues provides a comprehensive view of epigenetic differences among tissues.

ENHANCER OF ZESTE 2 POLYCOMB REPRESSIVE COMPLEX (EZH2): A histone-lysine N-methyltransferase enzyme involved in histone methylation and maintaining transcriptional repression over successive cell generations. Mutation or overexpression of EZH2 has been associated with a variety of cancers, and its inhibition has been shown to be responsible for suppressing tumor development. Thus blocking EZH2 activity might slow tumor growth.

PROGRAMMED CELL DEATH PROTEIN 1 (PD-1): A cell-surface molecule that, when expressed on T cells, pro-B cells, and myeloid-derived dendritic cells, leads to negative regulation of proliferation and activity. PD-1 and its ligand, programmed death-ligand 1 (PD-L1), play a major role in suppressing the immune system by sending an inhibitory signal to reduce T-cell activity.

SUPERENHANCER: A region of the mammalian genome comprising multiple enhancers in close genomic proximity to drive transcription of genes critical for cell-type specification and function.

TEN-ELEVEN TRANSLOCATION (TET): Proteins involved in regulation of DNA methylation and transcription named for a common translocation between chromosomes 10 and 11, creating an MLL-TET1 fusion protein found in patients with several forms of cancer.

TOPOLOGICALLY ASSOCIATED DOMAIN (TAD): Linear units of chromatin that fold as discrete 3-dimensional structures and tend to favor internal, rather than external, physical chromatin interactions. It has been shown that disruption of TADs leads to disease because of the transformation of 3-dimensional organization and thus disrupting gene regulation.

WHOLE-GENOME BISULFITE SEQUENCING (WGBS): A type of next-generation sequencing technology that has enabled genome-wide analysis of 5-methylcytosine (5mC) nucleotides at single nucleotide resolution by treating DNA with sodium bisulfite before sequencing. Sodium bisulfite converts unmethylated cytosines into uracil, and unconverted cytosines are methylated. This allows for detection of unmethylated cytosines to be detected as thymines after sequencing. Methylation of DNA at cytosine nucleotides forms 5mC, which affects various cellular processes involving gene expression and chromatin remodeling.

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