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The molecular basis of the memory T cell response: differential gene expression and its epigenetic regulation

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Abstract

How the immune system remembers a previous encounter with a pathogen and responds more efficiently to a subsequent encounter has been one of the central enigmas for immunologists for over a century. The identification of pathogen-specific memory lymphocytes that arise after an infection provided a cellular basis for immunological memory. But the molecular mechanisms of immunological memory remain only partially understood. The emerging evidence suggests that epigenetic changes have a key role in controlling the distinct transcriptional profiles of memory lymphocytes and thus in shaping their function. In this Review, we summarize the recent progress that has been made in assessing the differential gene expression and chromatin modifications in memory $CD4^+$ and $CD8^+$ T cells, and we present our current understanding of the molecular basis of memory T cell function.

Immunological memory is one of the defining features of the adaptive immune response and its induction is the basis for immunization and vaccination[1–2]. The cellular foundation of immunological memory resides in the existence of memory lymphocytes, which carry the 'memory' of a previous exposure to an antigen, along with an altered and enhanced functional capacity. Although immunological memory has been intensively studied in the past few decades, the mechanisms underlying the generation and maintenance of memory lymphocytes during and after an immune response remain only partially understood. Nevertheless, the key features of memory lymphocytes that make these cells distinct from naive lymphocytes are known; specifically, memory lymphocytes are longer lived and have a reduced activation threshold and enhanced effector functions.

The distinct features of a cell are determined principally by its transcriptional profile. Accordingly, the underlying basis of the acquired functions of memory lymphocytes is, primarily, their distinct patterns of gene expression. How memory lymphocytes acquire their gene expression patterns is not fully known, but recent advances in identifying unique patterns of gene expression and `epigenetic regulation` define a `chromatin` state that

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underpins memory T cell gene expression and function. In this Review, we summarize the current knowledge of gene expression profiles and kinetics in memory T cells during activation and differentiation, and we discuss the epigenetic features that are associated with the unique transcriptional profiles of memory T cells.

Gene expression in memory T cells

Memory T cells differentiate from naive T cells after antigenic stimulation and exhibit a sustained and enhanced response to the primary stimulus (antigen) during a subsequent encounter. Memory T cell populations are heterogeneous and can be divided into two main subsets: `central memory T cells` (T_{CM} cells) and `effector memory T cells` (T_{EM} cells)[3,4]. T_{CM} cells express CD62L (also known as L-selectin) and CC-chemokine receptor 7 (CCR7), circulate in lymphoid organs and have the stem cell-like ability to differentiate and proliferate after receiving proper signals (from antigens or cytokines). T_{EM} cells do not express CD62L or CCR7, and they circulate in non-lymphoid tissues. In addition, T_{EM} cells express high levels of effector molecules, such as cytokines (in the case of $CD4^+$ T_{EM} cells) or granzyme B and perform (in the case of $CD8^+$ T_{EM} cells), enabling them to perform rapid effector functions following activation.

Most human studies have defined memory T cells by their selective expression of CD45RA, CD45RO, CD62L and/or CCR7, as well as other cell-surface markers, whereas memory T cells in mice are often studied using antigen-specific approaches. Despite differences in the approaches used to study human and mouse memory T cell responses, the core transcriptional features of memory T cells are shared.

The development of `microarray` technology has revolutionized gene expression analysis and provided a powerful tool for the genome-wide evaluation of cellular gene expression profiles[5,6]. Thus, the transcriptional identity of a cell can be defined by a set of signature genes and can be used for comparison between different types of cells, as well as between the same types of cells at different stages of differentiation. Analyses of gene expression profiles of memory T cells have been reported for both $CD4^+$ and $CD8^+$ T cells from humans and mice[7–17]. Because of the differences in experimental platforms and analysis tools (as well as in specific details such as the mouse strains and the criteria used to identify memory T cells) across various microarray experiments, considerable variations exist in the details of the gene expression data. Here, we analyse published findings on $CD4^+$ and $CD8^+$ memory T cells from both humans and mice using the deposited microarray datasets from the Gene Expression Omnibus (GEO) database hosted by the National Center for Biotechnology Information (NCBI) (dataset numbers GSE24759, GSE22880, GSE14422, GSE23663, GSE24151, GSE26928, GSE32596, GSE21360 and GSE13743)[13–16,18–21]. We discuss transcriptional features that are shared between all memory T cells and compare them with those shared between $CD4^+$ and $CD8^+$ memory T cells and between T_{CM} and T_{EM} cells.

Global transcriptional profiles of memory T cells

The analysis of gene expression characteristics in memory T cells is often carried out by comparing these characteristics with the gene expression characteristics of precursor naive T

cells. Despite their substantial functional differences, naive and memory T cells share a great degree of similarity (~95%) in their overall gene expression profiles (FIG. 1). Indeed, only a few percent of their expressed genes (equivalent to a few hundred genes) are expressed at significantly different levels between naive and memory T cells[13–16,18–21]. Although genes that are highly expressed only in naive T cells are likely to be important in understanding the function of memory T cells, the roles of most of these genes are poorly characterized and their precise contributions to memory T cell function are not all clear. Therefore, we focus here on well-characterized genes that are highly expressed in memory T cells. Broadly speaking, these genes can be divided into different functional classes: genes with known immune function; genes that promote T cell survival and homeostasis; and genes with multiple or undefined functions (TABLE 1).

The genes that are highly expressed in memory T cells and that are involved in immune function can be further divided into several subgroups. First, there are genes that regulate the activation of T cells, including MHC class II genes (*HLA-DRA*, *HLA-DRB1*, *HLA-DPA1* and *HLA-DPB1*). The second subgroup comprises genes involved in the migration of T cells, including adhesion molecule and chemokine receptor genes, such as *CCR5*, *CCR6*, CXC-chemokine receptor 3 (*CXCR3*) and *CXCR5*. The third subgroup of genes is involved in intracellular signalling; these genes include mitogen-activated protein kinase kinase kinase 5 (*MAP3K5*), dual specificity phosphatase 4 (*DUSP4*), regulator of G-protein signalling 1 (*RGS1*) and S100 calcium-binding protein A4 (*S100A4*). Finally, the fourth subgroup includes effector molecule genes, such as granzyme A (*GZMA*) and *GZMK*.

The genes involved in memory T cell survival and homeostasis can be further separated into two subgroups. The first subgroup includes cytokine and chemokine genes, such as CC-chemokine ligand 5 (*CCL5*), interferon-γ (*IFNG*) and tumour necrosis factor (*TNF*). Genes in the second subgroup encode receptors, such as interleukin receptors (*IL2RB* and *IL10RA*), lectins (*LGALS1* and *LGALS3*), tumour necrosis factor receptor superfamily, member IB (*TNFRSF1B*), *CD74* and *FAS*.

The third class of genes that are highly expressed in memory T cells includes genes encoding transcriptional regulators, which serve an array of diverse functions. Such genes include *MAF*, thymocyte selection-associated high mobility group box (TOX) and *TBX21* (which encodes T-bet).

Comparing human $CD4^+$ and $CD8^+$ memory T cells, there seem to be more differentially expressed genes in $CD8^+$ memory T cells than in $CD4^+$ memory T cells (FIG. 1). Analysing these differentially expressed genes shows that $CD8^+$ memory T cells highly express genes that encode key effector molecules of cytotoxicity and killing — such as granzymes (*GZMB* and *GZMH*), cathepsin W (*CTSW*), perform 1 (*PRF1*), *CD160*, *CD244* and killer cell lectin-like receptors (*KLRC1*, *BCLRF1* and *KLRG1*) — as well as genes that encode chemokines (*CCL5*), complement factor H (*CFH*), interleukin receptors (*IL2RB*) and a master transcription factor (*TBX21*) (TABLE 1). These highly expressed genes make perfect sense for explaining the enhanced cytotoxic functions of $CD8^+$ memory T cells.

Although most of the highly expressed genes in memory T cells are shared between T_{CM} and T_{EM} cells, some genes are more highly expressed in either T_{CM} or T_{EM} cells. *CCR2*, *LGALS1*, *LGALS3*, the MHC class II genes *HLA-DPB1*, *HLA-DQA1*, *HLA-DRA*, *HLA-DRB5* and *HLA-DRB6*, and integrin αM (*ITGAM*) are more abundantly expressed in human $CD4^+$ T_{EM} cells than in human $CD4^+$ T_{CM} cells (on the basis of analyses of the GSE24759 and GSE26928 datasets)[15,16]. Genes that are more abundantly expressed in human $CD8^+$ T_{EM} cells than in human $CD8^+$ T_{CM} cells include genes encoding cell-surface receptors — such as chemokine receptors (*CCR6* and *CCR9*), killer cell lectin-like receptors (*KLRC1*, *KLRD1*, *KLRF1* and *CD244*), integrins (*ITGA4* and *ITGAE*), interleukin receptors (*IL18R1* and *IL23R*) and the apoptosis inducer FAS ligand (*FASLG*) — as well as cytotoxic molecule genes (*GZMH*) and *IFNG* (on the basis of analyses of the GSE14422, GSE24759 and GSE23663 datasets)[13,16,21]. The highly expressed genes in T_{EM} cells explain the effector function and the characteristic migration and circulation of these cells.

Collectively, the identification of highly expressed genes in $CD4^+$ and $CD8^+$ memory T cells, as well as in T_{CM} and T_{EM} cells, at the genome-wide level offers a transcriptional basis for explaining some of the unique features of memory T cells and of individual memory T cell subsets, such as enhanced effector functions, unique circulation and homing patterns, and homeostasis. It is essential to further characterize the roles of the genes with unknown functions that are highly expressed in memory T cells. A better understanding of the function of these genes will certainly reveal new insights into different aspects of memory T cells, and will lead to the elucidation of how the transcriptional state of these genes contributes to the overall function of memory T cells.

Kinetic features of memory T cell gene expression

Based on the kinetics of gene expression before and after antigen-mediated T cell activation, genes that are highly expressed in memory T cells compared with naive T cells can be further divided into two main classes (FIG. 2). First, there are genes that are highly expressed by resting memory T cells but not by resting naive T cells[7,13–16,18–21]. Second, there are activation-induced genes that are upregulated more rapidly in activated memory T cells than in activated naive T cells. Expression of this second group of genes is similar between resting naive and memory T cells, but they are expressed at higher levels by activated memory T cells than by activated naive T cells; these genes are also called 'poised' genes[8,13,22].

In human $CD8^+$ memory T cells, the highly expressed genes include killer cell lectin-like receptor genes (*KLRC1*, *KLRF1* and *KLRG1*) and granzyme genes (*GZMA*, *GZMB*, *GZMH* and *GZMK*), which mediate the cytotoxic and killing functions of $CD8^+$ T cells[13]. Activation-induced poised genes in memory T cells include interleukin-3 (*IL3*), *IL5*, *IL10* and *IL21* and these genes provide a fast and robust effector function for memory T cells following activation[13].

These two distinct patterns of highly expressed genes in memory T cells based on expression kinetics provide an additional level of regulation to precisely control gene function in a time- and space-dependent manner. Although the signals that maintain the high-level expression of specific genes in resting memory T cells are not completely

understood, the functional role of the well-characterized genes discussed in the previous section is obvious for the enhanced effector functions, migration and homeostasis of memory T cells. The expression of the poised genes is tightly regulated in the resting state and after activation, indicating that the activities of these genes are only desired following T cell activation. Further characterization of the kinetic changes in the expression of genes that are highly expressed in memory T cells will offer new insights into how memory T cells are generated and maintained from a transcriptional perspective.

Epigenetic regulation in memory T cells

The sustained and distinct patterns of gene expression in cells are believed to be controlled by epigenetic changes at the chromatin level[23,24]. The molecular nature and scope of the epigenetic changes during memory T cell development are currently under intensive study[25], and chemical modifications of DNA and histones are the most commonly studied epigenetic changes.

Epigenetic markers and their roles in chromatin state and transcription

Chromatin structure is dynamic and differs between one region (or gene) on a chromosome and another[26–28]. There are two basic states of chromatin: an open chromatin state that is accessible to DNA-binding proteins (such as transcription factors, transcriptional activators or repressors, and RNA polymerase) and thus facilitates active transcription; and a closed chromatin (`heterochromatin`) state that lacks accessibility for the transcriptional machinery and thus is associated with gene silencing. Several epigenetic markers have been identified in association with specific chromatin states and transcription levels[23] (TABLE 2).

Covalent modifications of DNA, in particular methylation, have been associated with chromatin states and transcriptional activity[29]. Methylation of cytosines within DNA occurs predominantly at clusters of CpG dinudeotides (known as CpG islands). It has been shown that approximately 70% of annotated gene promoters are associated with a CpG island[30]. Low-level or no methylation of CpG islands is associated with open chromatin and active gene transcription, whereas a high level of CpG island methylation is linked to heterochromatin and transcriptional silencing. Some CpG islands are located at a distance from transcription start sites (for example, at enhancers), and these sites can also influence transcription[31].

Covalent modifications of the amino-terminal tails of histones (namely, histones H2A, H2B, H3 and H4) have also been shown to regulate the chromatin state and transcription[26,27]. Histone modifications that are associated with open chromatin include: acetylation of H2A, H2B, H3 lysine 9 (H3K9), H3K14, H4K5 and H4K16; methylation of H3K4, H3K36 and H3K79; phosphorylation of H3 threonine 3 (H3T3), H3 serine 10 (H3S10) and H3S28; and ubiquitylation of H2BK120. Histone modifications that are associated with closed chromatin include: methylation of H3K9, H3K27 and H4K20; ubiquitylation of H2AK119; and sumoylation of H2BK6 or H2BK7 and H2AK126.

Conventional chromatin immunoprecipitation (ChIP) and reverse transcription PCR (RT-PCR) assays allow assessment of the chromatin state and transcription of selected genes, yielding valuable information on their chromatin state and transcriptional status during T cell differentiation[32,33]. Furthermore, the development of the ChIP-seq technique, which is a combination of ChIP and high-throughput sequencing methods, allows the direct assessment of the chromatin state, in terms of histone modifications and DNA-binding proteins, at the whole-genome level[34]. Combining results from global gene expression analysis methods (that is, microarrays and RNA sequencing) with ChIP-seq data makes it feasible for the first time to produce a genome-wide picture of the chromatin state and transcriptional activity of a population of cells.

DNA methylation and transcription in memory T cells

DNA methylation of CpG islands in genes encoding cytokines and their receptors, effector molecules, and their regulators has been long studied in memory T cells[35–41]. In $CD4^+$ memory T cells, *CCR6*, RAR-related orphan receptor C (*RORC*) and genes encoding ligands for P-selectin and E-selectin have been shown to be hypomethylated, in contrast to the hypermethylated states of these loci in naive $CD4^+$ T cells[35–37]. Correspondingly, higher expression of *CCR6* and *RORC* is found in memory T cells than in naive T cells. In $CD8^+$ memory T cells, low levels of DNA methylation at the *IFNG* and *IL2* loci and concomitant high protein expression levels have been observed after activation[38–40]. Furthermore, rapid DNA demethylation in the promoters of these genes occurs in $CD8^+$ memory T cells but not $CD8^+$ naive T cells after activation[39]. Such dynamic changes in DNA methylation are also found at the programmed cell death 1 (*Pdcd1*) gene locus (which encodes a key regulator of cell proliferation and exhaustion) during naive to effector to memory $CD8^+$ T cell differentiation following viral infection[42].

As the DNA methylation status is stable and passes from a parental cell to its descendants after memory T cell division[38], the feature of enhanced or poised gene expression is retained in the descendants of memory T cells. Thus, DNA methylation-related chromatin changes provide a transcriptional basis for the enhanced effector response, a key feature of memory T cells. In addition, the DNA methylation status of a cell death-related gene (*Noxa*; also known as *Pmaip1*) has been shown to influence the survival of $CD4^+$ memory T cells[41]; indeed, the repression of *Noxa* expression by DNA methylation is necessary for the survival of these cells.

It is evident that DNA methylation has a key role in the regulation of gene expression in memory T cells. However, the full scope of the involvement of DNA methylation in the differential gene expression of memory T cells remains to be determined. A recent genome-wide analysis of changes in DNA methylation during immune cell differentiation observed a robust change in DNA methylation during early lineage differentiation but only a minor change during later stages of differentiation[43]. It is therefore possible that the changes in DNA methylation status during naive to memory T cell differentiation might be limited to key genes of memory T cell function, such as cytokines and growth-related molecules and their regulators.

Histone modifications and gene expression in memory T cells

Similarly to DNA methylation, histone acetylation has been analysed in cytokine genes during $CD4^+$ memory T cell differentiation[32,44]. Histone hyperacetylation is associated with an active chromatin conformation and is found at the promoters of *IFNG* and *IL4* in memory T helper 1 (T_H1) and T_H2 cells, respectively[45,46]. Similarly, histone hyperacetylation is observed at the promoters of genes encoding cytokines (such as IFNγ), effector molecules (such as granzyme B and perform 1), and their regulators in $CD8^+$ memory T cells[40,47–49]. More importantly, induced hyperacetylation or hypoacetylation at these gene loci results in an increase or decrease, respectively, in their expression in $CD8^+$ T cells. These findings show that histone acetylation at these effector gene loci is necessary for their enhanced expression in memory T cells, thus providing evidence that histone acetylation state regulates memory T cell function.

Analyses of genome-wide histone methylation (specifically, trimethylation of H3K4 (H3K4me3) and H3K27me3) and gene expression show a general correlation between gene expression and the distribution of histone methylation. Indeed, H3K4me3 is positively correlated and H3K27me3 is negatively correlated with gene expression[13–50]. Further analysis reveals four distinct states that are determined by histone methylation and that differentially control gene expression: active, poised, bivalent and repressed (FIG. 3). During the differentiation of a $CD4^+$ naive T cell to an effector T cell, the chromatin state is altered at the gene locus encoding the key transcription factor that controls the differentiation of the particular effector T cell subset (that is, *TBX21* for T_H1 cells, *GATA3* for T_H2 cells, *RORC* for T_H17 cells and *FOXP3* for regulatory T cells), such that the original closed chromatin state (high levels of H3K27me3 and low levels of H3K4me3) changes to an open chromatin state (high levels of H3K4me3 and low levels of H3K27me3)[33,50]. In T_H2-polarized $CD4^+$ memory T cells, methylation of histones H3 and H4 at the *IL4* and *IL13* gene loci[46] is observed, together with H3K4 methylation at the *GATA3* locus (which encodes the master transcriptional regulator of IL-4 expression)[51]. Furthermore, the histone methyltransferase HRX (which is encoded by *MLL* and catalyses histone H3 and H4 methylation) is involved in the production of T_H2 cell-associated cytokines. Decreased HRX expression results in reduced H3K4me2 levels at the *GATA3* and *IL4* gene loci, and thus in decreased expression of GATA3 and IL-4, in $CD4^+$ memory T cells[52]. Collectively, these findings show that histone methylation influences open and closed chromatin states and thereby regulates the differential expression of key genes in $CD4^+$ memory T cells.

Analyses of histone methylation (specifically, H3K4me3 and H3K27me3) in $CD8^+$ memory T cells in humans[13] and mice[53] have shown that genes associated with effector functions (such as *PRDM1, KLRG1, Ifng* and *Gzmb*) have high levels of H3K4me3 and low levels of H3K27me3. It has also been shown that certain genes — such as inhibitor of DNA binding 2 (*ID2*), which has a key role in the survival of $CD8^+$ memory T cells following their activation[54] — have an open chromatin state but low mRNA levels in resting $CD8^+$ memory T cells (which is termed a poised state)[13]. This finding reveals a molecular basis for the rapid and enhanced expression of poised genes following the activation of memory T cells. In addition, bivalent chromatin — which contains histone modifications that are associated

with both open and closed chromatin (that is, high levels of both H3K4me3 and H3K27me3 at the same region) — is first found in stem cells and is believed to be involved in the differentiation of stem cells after they receive a differentiation signal[55,56]. Bivalent chromatin has also been observed at several gene loci in memory T cells[13]. For example, *KIAA1804* (mixed lineage kinase 4), which is involved in `Toll-like receptor` 4 (TLR4) signalling[57], has a bivalent chromatin state in resting $CD8^+$ memory T cells but changes to an open chromatin state and is expressed at higher levels after $CD8^+$ memory T cell activation[13].

Thus, genes with a bivalent chromatin state in resting memory T cells could more rapidly assume an open chromatin state and initiate transcription in response to T cell activation than genes in a closed chromatin state. It is clear that the four epigenetic states mentioned above (that is, active, poised, bivalent and repressed) provide a means to regulate gene expression to facilitate the function of memory T cells in the resting state and after antigenic activation. Whether the particular states at specific gene loci are fixed properties of memory T cells or can change in memory T cells is currently unclear. It is conceivable that variations in these epigenetic states could be a source of the heterogeneity of memory T cell populations. In the effector phase of $CD4^+$ T cell differentiation, there is increasing evidence of plasticity among the different T_H cell subsets[58,59]. However, it remains to be determined how stable or flexible these specific states of chromatin are at individual gene loci in memory T cells during their homeostasis and after activation.

Complexity of epigenetic changes and their meaning and regulation

In memory T cells, the role of epigenetic changes that involve other post-translational histone modifications (such as ubiquitylation and sumoylation) or the remodelling of nucleosomes within chromatin remains to be elucidated. For the known modifications, it becomes apparent that the same chromatin state can be influenced by several different epigenetic changes; for example, an open chromatin state is influenced by DNA hypomethylation, histone acetylation and certain types of histone methylation and, therefore, is a result of combinatorial changes in multiple modifications[60]. Intriguingly, not all open chromatin regions with active gene transcription have identical patterns of histone modifications[13]. It is currently unclear whether the particular combinatorial patterns of epigenetic modifications are unique for specific genes, for specific types of cell and/or for specific stages of differentiation, and whether such differences or redundancies in histone modifications contribute to the heterogeneity of memory T cells. Therefore, further study is needed to characterize different patterns of epigenetic changes and to understand the meanings of particular patterns and their roles in the regulation of differential gene expression in memory T cells. In addition, to facilitate chromatin and gene expression studies with greater resolution, it will be necessary to develop sensitive methods that are capable of analysing the epigenetic changes at the single-cell and single-chromosome levels.

How the epigenetic changes in memory T cells are established and maintained is not known. Current evidence suggests that a series of different steps may be needed to establish an open or closed chromatin conformation. For example, it has been shown that the prior DNA methylation status serves as a basis for recruiting histone-modifying enzymes, such as the

H3K4 methyl-transferase SETD1, to regulate transcription[61]. In addition, enzymes that catalyse histone acetylation and deacetylation — histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively — are both targeted to transcribed regions of active genes (where the function of HDACs is to reset chromatin by removing acetylation[62]) and are both intricately regulated by histone methyltransferases. The poised genes that are primed by HRX-mediated methylation of histone H3K4 are further modified by a dynamic cycle of acetylation and deacetylation through transient HAT and HDAC binding[52]. In repressed genes, trimethylation of H3K27 is mediated by the histone methyltransferase EZH2 (enhancer of zeste homologue 2) as part of Polycomb repressive complex 2 (PRC2). The activity of EZH2 in mediating trimethylation of H3K4 is dependent on the activity of HDACs, as limited HDAC activity markedly reduces H3K4me3 levels and gene transcription[63]. The current challenges are to elucidate the sequences of events that are involved in establishing a particular chromatin state at the loci of differentially expressed genes during memory T cell formation and in its subsequent maintenance, and to determine whether the sequence of epigenetic events that establishes a particular chromatin state is uniform for all gene loci or varies among the different gene loci in memory T cells.

During memory T cell formation, epigenetic changes such as histone acetylation and methylation are thought to be initiated during the effector phase and to persist in the memory phase in the absence of antigenic stimulation[64]. Therefore, the process of epigenetic modification in memory T cells can be conceptually divided into two stages. First, chromatin changes are established in the loci of genes encoding proteins with effector functions (such as cytokines and cytotoxic molecules) during the differentiation of naive T cells into effector T cells. Second, during the transition of a T cell from an effector to a memory phenotype, the chromatin status is maintained at these gene loci, and this maintenance is accompanied by additional modifications in the chromatin states of genes that have a dominant function in effector T cells and the establishment of new chromatin states in gene loci that are differentially expressed in memory T cells. Elucidation of the precise epigenetic processes at these two stages during memory T cell formation is essential not only for understanding how the epigenetic changes are established and maintained in memory T cells, but also for providing the molecular benchmarks to evaluate the long-lasting protectiveness of an immune response.

Clinical implications

Knowledge of T cell transcription signatures and the epigenetic modifications of these signature genes has significantly enhanced our understanding of the differences between memory T cells and naive T cells at the transcriptional level, and of the chromatin basis for the enhanced transcriptional and functional activities of memory T cells. An effective immune response to a pathogen or a vaccine yields competent effector and memory T cells. The quantity and quality of T_{EM} and T_{CM} cells will determine how well the host is protected from a subsequent encounter with the same pathogen. As the adaptive immune response is highly heterogeneous for different pathogens and in different individuals, the ability to assess the immune function of an individual before and after vaccination is crucial for determining the success of a vaccination. It has been shown that assessing an immune response at the transcriptional level by examining the expression of immune-relevant genes

using a systems biology approach provides a comprehensive portrait of an immune response[65]. By evaluating the expression kinetics of key genes (including those encoding cytokines, effector molecules and their master transcriptional regulators), the outcome of an immune response to a vaccine can be predicted. Thus, it is possible that the level of immune competency can be evaluated based on the transcriptional status and epigenetic features of specific signature genes, in combination with cellular characterization of antigen-specific B and T cells. This information before vaccination could be used to select the type and dosage of the vaccine, and information generated after vaccination would allow for the measurement of the T cell response and would enable us to predict whether a long-lasting memory T cell response is achievable and also to avoid unfruitful or even harmful consequences of vaccination. Furthermore, a better understanding of the immune response of the host could in turn guide the design of vaccines.

Considering the role of epigenetic changes in the gene expression patterns and enhanced function of memory T cells, it is not surprising that there can be detrimental consequences to the body when the epigenetic changes occur at a gene or several genes at an improper time or in an autoreactive T cell. A recent report shows that there are alterations in the DNA methylation and histone acetylation status of T cells from patients with the autoimmune disease systemic lupus erythematosus (SLE), which has a much higher prevalence in women[66,67]. For example, the gene encoding CD40 ligand (*CD40LG*), which is involved in B cell-T cell interactions, is demethylated in $CD4^+$ T cells from women with SLE, but not in $CD4^+$ T cells from men with SLE, and this correlates with an increased expression of *CD40LG* in female patients. Intriguingly, as *CD40LG* is located on the X chromosome, its demethylated status on the inactive X chromosome is associated with its enhanced expression and therefore may contribute to the striking female predilection of SLE[66]. Another study described global histone hypoacetylation in the absence of histone H3K4 methylation in $CD4^+$ T cells from patients with SLE compared with $CD4^+$ T cells from healthy controls[67]. If these autoreactive T cells develop a chromatin landscape of memory T cells, the disease state will be persistent and/or will deteriorate. Clearly, more studies are needed to understand the scope of epigenetic changes in autoreactive T cells. Furthermore, a better understanding of the process of generating epigenetic changes in normal memory T cells may have therapeutic applications in preventing autoreactive T cells from becoming memory T cells.

Conclusion

The identification of differentially expressed genes and their epigenetic regulation provides a chromatin basis for explaining the transcriptional changes and functions of memory T cells. Future studies should further elucidate the functions of many of the highly expressed genes in memory T cells, as well as in naive T cells, to enable us to gain a complete appreciation of memory T cell formation, maintenance and function. In addition, we should focus on how specific epigenetic changes are established and maintained in memory T cells and on how numerous transcription factors and histone-modifying enzymes are coordinated at a specific gene locus and at the correct time during naive to memory T cell differentiation and memory T cell activation. It will also be important to determine whether dysregulation

of epigenetic changes contributes to the altered function of the immune system in degenerative processes, such as autoimmunity and ageing.

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Glossary

Epigenetic regulation	The modifications on DNA, histones and other targets that collectively determine a stable phenotype without altering the DNA sequence. Epigenetic changes can pass from the parental cells to their offspring and provide a molecular basis for cellular memory.
Chromatin	The combination of DNA, histones and other proteins that comprises eukaryotic chromosomes. The basic repeating unit of chromatin is the nucleosome, which consists of an octamer of histone proteins around which ~ 146 base pairs of DNA is wound.
Central memory T cells (T_{CM} cells)	Antigen-experienced T cells that lack immediate effector function but can mediate rapid recall responses. They also rapidly develop the phenotype and function of effector memory T cells after re-stimulation with antigen. T_{CM} cells retain the migratory properties of naive T cells and therefore circulate through the secondary lymphoid organs.
Effector memory T cells (T_{EM} cells)	Terminally differentiated T cells that lack lymph node-homing receptors but express receptors that enable them to home to inflamed tissues. T_{EM} cells can exert immediate effector functions without the need for further differentiation.
Microarray	A tool for measuring gene transcription. Its use involves the hybridization of fluorescently labelled cDNA prepared from a cell or tissue of interest with thousands of known oligonucleotides or cDNAs dotted on glass slides or other surfaces. The known DNA ideally represents all of the expressed genes in the species.
Heterochromatin	High-density regions in the nucleus that are thought to contain compacted chromatin structures associated with silent genes.
Chromatin immunoprecipitation	A technique that uses antibodies specific for transcription factors or other DNA-binding proteins to precipitate associated DNA sequences from chromatin to study their functional relationship.

Reverse transcription PCR	A type of PCR in which RNA is converted into complementary DNA (cDNA), which is then amplified.
ChIP-seq	A technique in which chromatin immunoprecipitation (ChIP) is followed by high-throughput sequencing to generate a genome-wide distribution map of protein–DNA interactions. This technique can be used to measure transcription factor binding and histone modifications.
Toll-like receptor (TLR)	A member of a family of receptors that are homologous to Drosophila melanogaster Toll. TLRs recognize conserved molecular patterns that are unique to microorganisms. The lipopolysaccharide component of bacterial cell walls is one such ligand. TLRs can also recognize mammalian components and contribute to autoimmunity.

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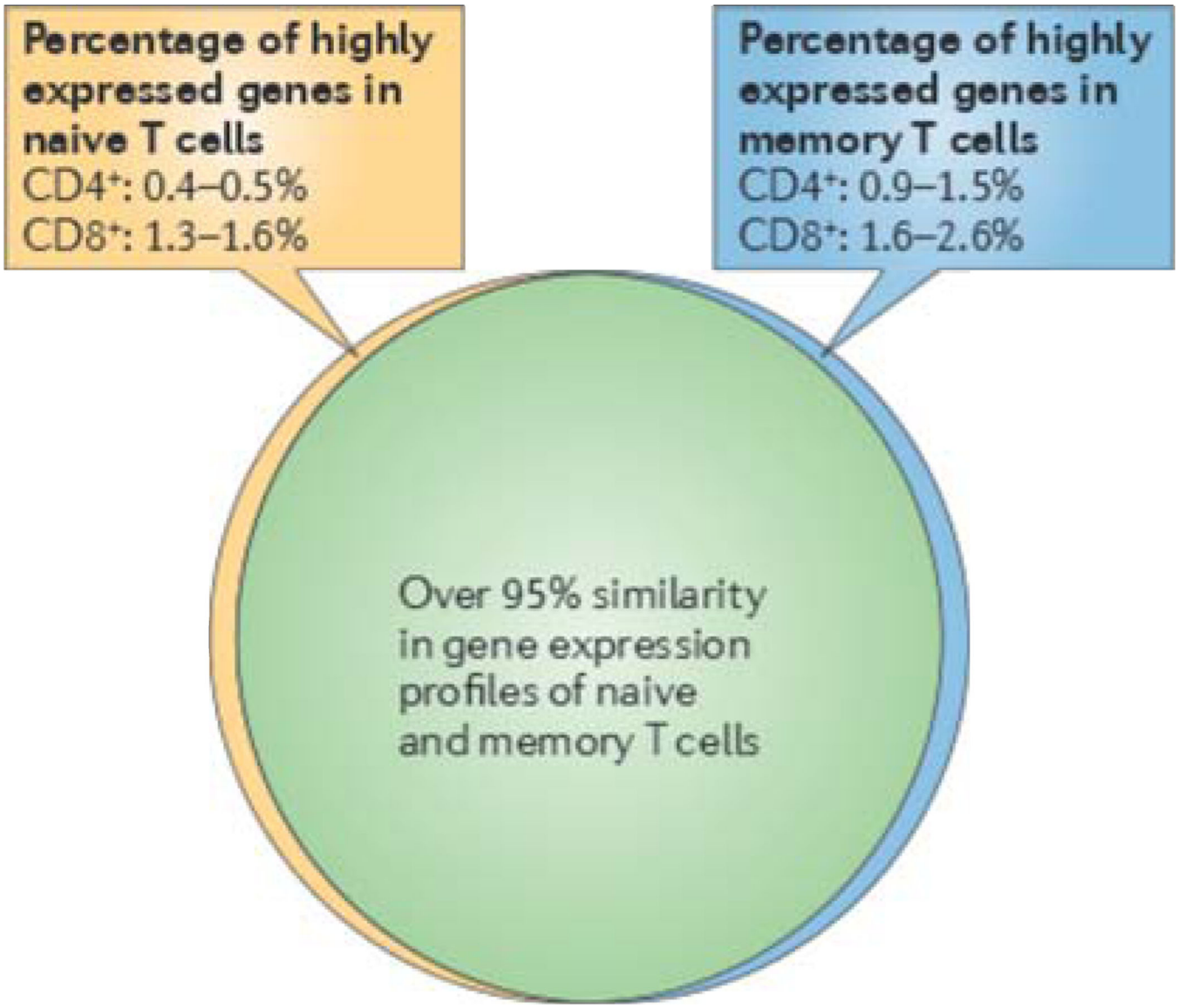

Figure 1. Comparison of overall gene expression in naive and memory T cells

The numbers in this figure are based on the analysis of nine published or deposited datasets from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (dataset numbers GSE24759, GSE22880, GSE14422, GSE23663, GSE24151, GSE26928, GSE32596, GSE21360 and GSE13743). These datasets describe the differences in gene expression between naive and memory $CD4^+$ and $CD8^+$ T cells from humans and mice. Because different array analyses have different numbers of annotated genes, the genes that are expressed significantly more highly (expression difference fold > 2.0 and $P < 0.05$) in naive or memory T cells are presented as a percentage of the total number of genes analysed. The ranges reflect the lowest to the highest percentages in the nine datasets analysed here. In general, fewer genes are differentially expressed between naive and memory $CD4^+$ T cells than between naive and memory $CD8^+$ T cells.

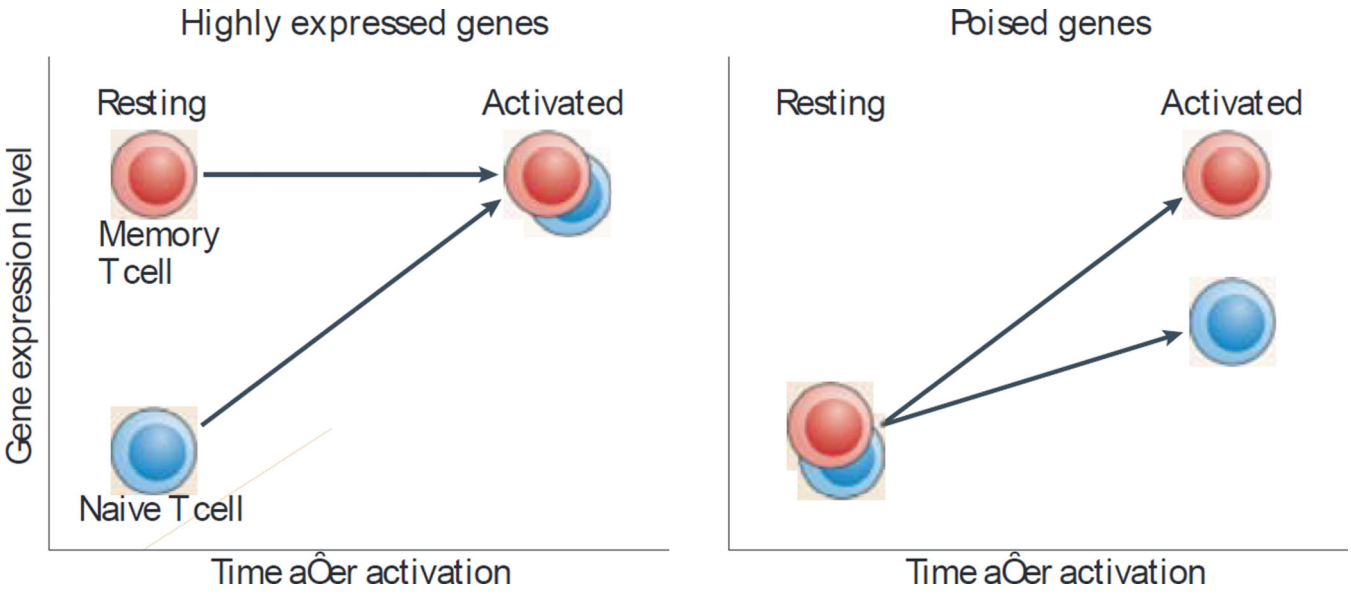

Figure 2. Types of genes that are differentially expressed in memory T cells based on their expression kinetics before and after T cell activation

There are two main kinetic patterns of expression for genes that are expressed at higher levels in memory T cells than in naive T cells. First, there are genes that are highly expressed in resting memory T cells compared with resting naive T cells. These highly expressed genes in resting memory T cells include genes involved in migration, homeostasis and readiness for activation. Second, there are genes that are highly expressed only after the activation of memory T cells; these genes are termed poised genes. Such poised genes are tightly regulated when the T cell is in the resting state but are rapidly induced after T cell activation. It is apparent that the function of these poised genes is not desired in the resting state, and therefore they are minimally expressed. These two patterns of expression for genes that are highly expressed in memory T cells show that the expression of such genes is precisely controlled in a time- and space-dependent manner to fulfil the function of memory T cells.

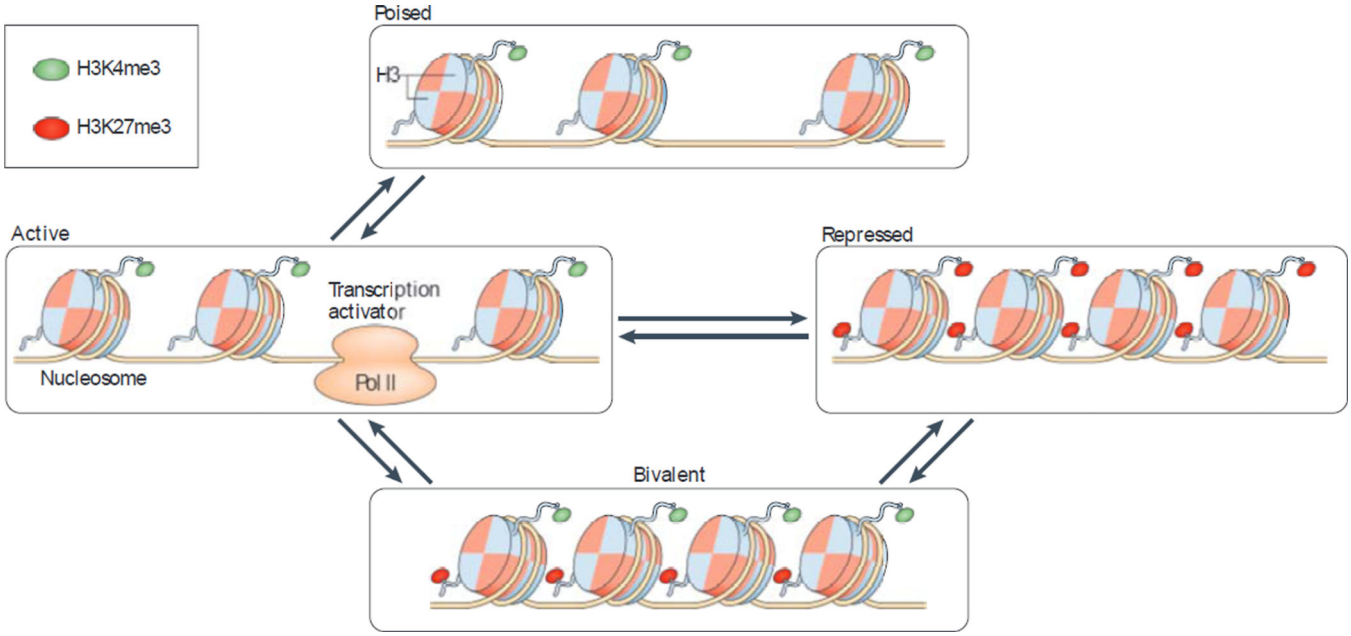

Figure 3. The chromatin basis for differential gene expression in memory T cells involves histone methylation

There are four distinct modes of relationship between histone methylation and gene expression in memory T cells: active, poised, bivalent and repressed. In the active mode, the gene locus has an open chromatin state, which is indicated by high levels of trimethylation at histone H3 lysine 4 (H3K4me3; an activating modification), and there is active gene transcription (indicated by the presence of the transcription activator). In the poised mode, the gene locus has an open chromatin state, similar to that of the active mode, but there is no active gene transcription in resting memory T cells. However, following T cell activation, the transcription of genes in the poised mode can be rapidly initiated. Genes in the bivalent mode contain high levels of both H3K4me3 and the repressive modification H3K27me3 at their loci. Such a chromatin state can change in either direction (to an open or closed state) after T cell activation, and this is followed by the initiation or repression of gene transcription. Genes with a repressed chromatin state contain low levels of H3K4me3 but high levels of H3K27me3 at their loci, and thus their transcription is repressed. The unique chromatin landscape of memory T cells provides a structural basis for the differential gene expression and function of memory T cells. Pol II, RNA polymerase II.

Table 1

Chromatin states of selected genes that are highly expressed by human memory T cells*

Gene symbol	Gene name	Chromatin state‡	Memory T cell subset§	Functional subgroup
Immune function				
CD58	CD58 molecule	Open	$CD4^+ \approx CD8^+$	Activation
CLECL1	C-type lectin-like 1	Open	$CD4^+ \approx CD8^+$	Activation
HLA-DRA	Major histocompatibility complex, class II, DR alpha	Open	$CD4^+ \approx CD8^+$	Activation
HLA-DRB1	Major histocompatibility complex, class II, DR beta 1	Open	$CD4^+ \approx CD8^+$	Activation
SLAMF1	Signalling lymphocytic activation molecule family member 1	Open	$CD4^+ \approx CD8^+$	Activation
HLA-DPA1	Major histocompatibility complex, class II, DP alpha 1	Open	$CD8^+ > CD4^+$	Activation
HLA-DPB1	Major histocompatibility complex, class II, DP beta 1	Open	$CD8^+$	Activation
CD63	CD63 molecule	Open	$CD4^+ \approx CD8^+$	Adhesion or migration
CXCR3	CXC-chemokine receptor 3	Open	$CD4^+ \approx CD8^+$	Adhesion or migration
CXCR5	CXC-chemokine receptor 5	Open	$CD4^+ \approx CD8^+$	Adhesion or migration
CCR5	CC-chemokine receptor 5	Open	$CD8^+ > CD4^+$	Adhesion or migration
CCR6	CC-chemokine receptor 6	ND	$CD4^+$	Adhesion or migration
CX3CR1	CX_3C-chemokine receptor 1	Open	$CD8^+$	Adhesion or migration
DUSP4	Dual specificity phosphatase 4	Open	$CD4^+ \approx CD8^+$	Signalling
MAP3K5	Mitogen-activated protein kinase kinase kinase 5	Open	$CD4^+ \approx CD8^+$	Signalling
PTGER2	Prostaglandin E receptor 2	Open	$CD4^+ \approx CD8^+$	Signalling
RGS1	Regulator of G-protein signalling 1	Open	$CD4^+ \approx CD8^+$	Signalling
S100A4	S100 calcium-binding protein A4	Open	$CD4^+ \approx CD8^+$	Signalling
DUSP5	Dual specificity phosphatase 5	ND	$CD4^+$	Signalling
LY96	Lymphocyte antigen 96	ND	$CD4^+$	Signalling
NOD2	Nucleotide-binding oligomerization domain-containing 2	ND	$CD4^+$	Signalling
STAM	Signal transducing adaptor molecule 1	ND	$CD4^+$	Signalling
CFH	Complement factor H	Open	$CD8^+ > CD4^+$	Effector molecule
GZMA	Granzyme A	Open	$CD8^+ > CD4^+$	Effector molecule
GZMK	Granzyme K	Open	$CD8^+ > CD4^+$	Effector molecule
GZMB	Granzyme B	Open	$CD8^+$	Effector molecule
GZMH	Granzyme H	Open	$CD8^+$	Effector molecule
CTSW	Cathepsin W	Open	$CD8^+$	Effector molecule
PRF1	Perforin 1	Open	$CD8^+$	Effector molecule
CD160	CD160 molecule	Open	$CD8^+$	Effector molecule

Gene symbol	Gene name	Chromatin state‡	Memory T cell subset§	Functional subgroup
Immune function				
CD244	CD244 molecule	Open	$CD8^+$	Effector molecule
NCR3	Natural cytotoxicity triggering receptor 3	Open	$CD8^+$	Killing-related function
KLRC1	Killer cell lectin-like receptor subfamily C, member 1	Open	$CD8^+$	Killing-related function
KLRF1	Killer cell lectin-like receptor subfamily F, member 1	Open	$CD8^+$	Killing-related function
KLRG1	Killer cell lectin-like receptor subfamily G, member 1	Open	$CD8^+$	Killing-related function
Survival and homeostasis				
IFNG	Interferon gamma	Open	$CD4^+ \approx CD8^+$	Cytokine or chemokine
TNF	Tumor necrosis factor	Open	$CD4^+ = CD8^+$	Cytokine or chemokine
CCL5	CC-chemokine ligand 5	Open	$CD8^+ > CD4^+$	Cytokine or chemokine
IL2RB	Interleukin-2 receptor beta	Open	$CD8^+ > CD4^+$	Receptor
IL10RA	Interleukin-10 receptor alpha	Open	$CD4^+ \approx CD8^+$	Receptor
LCALS1	Lectin, galactoside-binding, soluble, 1	Open	$CD4^+ \approx CD8^+$	Receptor
LGALS3	Lectin, galactoside-binding, soluble, 3	Open	$CD4^+ \approx CD8^+$	Receptor
TNFRSF1B	Tumor necrosis factor receptor superfamily, member IB	Open	$CD4^+ \approx CD8^+$	Receptor
CD74	CD74 molecule (HLA class II histocompatibility antigen gamma chain)	Open	$CD4^+ \approx CD8^+$	Receptor
FAS	FAS (TNF receptor superfamily, member 6)	Open	$CD4^+ \approx CD8^+$	Receptor
CDKN1A	Cyclin-dependent kinase inhibitor 1A	Open	$CD4^+ \approx CD8^+$	Receptor
Multiple or undefined functions				
MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homologue (avian)	Open	$CD4^+ \approx CD8^+$	Transcriptional regulator
TOX	Thymocyte selection-associated high mobility group box	Open	$CD4^+ \approx CD8^+$	Transcriptional regulator
TBX21	T-box 21 (encodes T-bet)	Open	$CD8^+ > CD4^+$	Transcriptional regulator

ND, not determined (no genome-wide data).

*The selection of highly expressed genes in human memory T cells was based on six datasets from the Gene Expression Omnibus (CEO) database (GSE14422, GSE26928, GSE24759, GSE24151, GSE22880 and GSE23663)[13,15,16,18,19,21]. The comparison between $CD4^+$ and $CD8^+$ memory T cells used the GSE24579 dataset[16]

‡Chromatin state was determined based on levels of trimethylation of histone H4 lysine 4 (H4K4me3) in $CD8^+$ memory T cells.

§$CD4^+ \approx CD8^+$, similarly highly expressed in both $CD4^+$ and $CD8^+$ memory T cells; $CD8^+ > CD4^+$, highly expressed in both $CD4^+$ and $CD8^+$ memory T cells but expressed significantly more highly in $CD8^+$ memory T cells; $CD4^+$, highly expressed only in $CD4^+$ memory T cells; $CD8^+$, highly expressed only in $CD8^+$ memory T cells.

Table 2

Chemical modifications of DNA and histones and their association with chromatin and transcription states

Target	Modification	Nucleotide or amino acid	Residue position	Chromatin state	Transcription state
DNA	Methylation	Cytosine (C)	CpG islands	Closed	Repressed
Histones	Acetylation	Lysine (K)	H2AK5, H2BK12, H2BK15, H3K9, H3K14, H3K18, H3K56, H4K5, H4K8, H4K13, H4K16	Open	Active
	Methylation	Arginine (R)	H3R17, H3R23, H4R3	Open	Active
	Methylation	Lysine (K)	H3K4, H3K36, H3K79	Open	Active
			H3K9, H3K27, H4K20	Closed	Repressed
	Phosphorylation	Serine (S) or threonine (T)	H3T3, H3S10, H3S28, H2BS14	Open	Active
	Sumoylation	Lysine (K)	H2AK126, H2BK6, H2BK7	Closed	Repressed
	Ubiquitylation	Lysine (K)	H2AK119	Closed	Repressed
			H2BK120	Open	Active