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Using epigenetics to define vaccine-induced memory T cells

Ben Youngblood^{1,2,3}, J Scott Hale^{1,2,3}, and Rama Akondy^{1,2,3}

¹Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA 30322, United States

²Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322, United States

³Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery, Emory University School of Medicine, Atlanta, GA 30322, United States

Abstract

Memory T cells generated from acute infection or vaccination have the potential to provide the host with life-long immunity against re-infection. Protection by memory T cells is achieved through their acquired ability to persist at anatomical sites of the primary infection as well as maintaining a heightened ability to recall effector functions. The maintenance of CD8 and CD4 T cell function in a state of readiness is key to life-long immunity and manifest through changes in transcriptional regulation. Yet, the ability to identify poised transcriptional programs at the maintenance stage of the response is lacking from most transcriptional profiling studies of memory T cells. Epigenetic profiling allows for the assessment of transcriptionally poised (promoters that are readily accessible for transcription) states of antigen-specific T cells without manipulation of the activation state of the cell. Here we review recent studies that have examined epigenetic programs of effector and memory T cell subsets. These reports demonstrate that acquisition of epigenetic programs during memory T cell differentiation to acute and chronic infections is coupled to, and potentially regulate, the cell's recall response. We discuss the usefulness of epigenetic profiling in characterizing T cell differentiation state and function for preclinical evaluation of vaccines and the current methodologies for single locus versus genome-wide epigenetic profiling.

Memory T cell differentiation to acute and chronic antigen exposure

Vaccine induced immunity to intracellular pathogens is mediated in part by a heightened recall of effector functions from antigen-specific CD4 and CD8 memory T cells. The recall response of memory T cells contribute to the rapid control of the cognate pathogen through their ability to home to sites of infection, lyse infected cells, and induce immune activation through the secretion of inflammatory cytokines. The expression of select cytokines and homing molecules by antigen-specific memory T cells is achieved through modifying naïve transcriptional regulatory programs in response to the strength and duration of the primary exposure to antigen (Figure 1a) [1–4]. As such, persistent primary exposure to antigen, as occurs during chronic infections or cancer, modifies the transcriptional program so that antigen-specific CD8 T cells progressively lose the ability to recall effector functions that would normally facilitate the efficient killing of infected or cancerous cells [5–7]. The eventual development of T-cell exhaustion leaves the host unable to control the chronic pathogen and presents a major challenge for generating protective vaccines. Fortunately, preexisting memory T cells of sufficient quantity and quality can expeditiously control

pathogens that would normally result in a chronic infection and avoid the exhaustive effects of prolonged antigen presentation. Vaccine strategies are now focused on generating a high quantity of functional pathogen-specific T cells that are poised to recall effector functions that also reside in, or rapidly traffic to, sites of infection.

In light of the protective potential of memory T cells, recent efforts have been focused on determining the specific functions of memory T cells that facilitate the control of chronic pathogens, and identifying which subset of memory T cells acquire the protective qualities. The ability of the immune system to partition distinct functions (proliferation, tissue homing, and effector molecule expression) into subsets of memory CD8 T cells facilitates the surveillance and recall of secondary effector functions and tailored to the primary pathogens initial site of infection or exposure. Characterization of proliferative potential, anatomical distribution, and recall of effector functions in CCR7 and CD62L^{lo} and ^{hi} memory T cell, first in human polyclonal CD4 and CD8 T cells populations, followed by a more extensive description using animal model systems, established a model for memory differentiation that delineates specific functions into distinct subsets [8,9]. Memory T cell heterogeneity is now broadly parsed into effector-memory (Tem), central-memory (Tcm), and recently described tissue-resident (Trm) subsets. Tem are phenotypically characterized by the downregulated expression of the homing molecules CD62L and CCR7 and circulate between nonlymphoid tissues and blood. Central memory T cells (Tcm) residing in the blood and secondary lymphoid tissues express CD62L and CCR7 and rapidly proliferate upon re-exposure to antigen (Figure 1b) [2,10–12]. The existence of a tissue resident memory T cell subset was revealed through xenografting and parabiosis experiments demonstrating that a distinct population of memory T cells are restricted from circulation (Figure 1b) [13,14]. Recent studies using two-photon microscopy further demonstrated that Trm cells indeed are restricted to the sites of primary antigen exposure following viral control [15–17] (Figure 1a). It has been demonstrated that the presence of Trm cells facilitates an immediate local recall response and is protective against pathogen reactivation (in the case of latently infected viruses) or re-infection at the site of their residence. The mechanism for the discriminatory residence of this subset of memory T cells is not well defined but the selective expression of integrins, including CD103, suggests that restricted circulation of Trm cells is controlled by a distinct compilation of adhesion molecules [3,18]. The anatomical restriction of these T cell subsets implies that the molecular determinants of homing molecule expression mediate memory T cell subset specialization. Given that these acquired tissue homing and residence properties are tightly coupled to the tropism and persistence of the pathogen, research efforts are now focused on establishing which subset of memory T cells provide protection against specific chronic infections or cancer with the goal of generating these specific populations through vaccination.

Vaccine generated protective T cells

To date, an effective vaccine against HIV remains elusive. A major hurdle in the development of a protective vaccine against HIV is defining the correlates of protection for evaluation of preclinical studies. Currently, the standard for assessing antigen-specific T cell function focuses on the multifunctional recall of cytokines and cytotoxic molecules following *in vitro* peptide stimulation. Indeed, enhanced T cell function correlates with reduction of HIV viremia in infected individuals who control the infection [19]. Yet this single criterion has been unable to fully explain the successes and failures of recent HIV vaccine trials designed to elicit cellular immunity [20,21]. The inability of these vaccines to generate protective cellular immunity to HIV begs the question as to whether or not vaccine generated memory T cells can control HIV. In support of T-cell based vaccine strategies, the Picker lab has recently demonstrated that a CMV-based vaccine can generate memory CD8 T cells of sufficient quantity and quality to control SIV infection of rhesus macaques, a

model for HIV infection [22••]. The protective population of memory CD8 T cells exhibits an effector-memory like phenotype, yet these cells reside in lymphoid as well as nonlymphoid tissues; therefore this pool of SIV-specific T cells also probably includes a central-memory population. In another study of vaccine generated CD8 T cells, the Watkins lab recently demonstrated that vaccination of rhesus macaques that have Mamu-B*08 MHC alleles (similar to human HLA-B*27 found in HIV elite controllers) can generate protective CD8 T cell based immunity against a lethal challenge of SIV [23]. These seminal studies provide compelling evidence that vaccine-generated T cell mediated HIV immunity is achievable. Yet, until the specific properties of the cellular response that resulted in protection are better defined, it may be difficult to translate these results into a HIV vaccine.

The above-described successes in generating protective SIV vaccines in nonhuman primates demonstrate that it is indeed possible to protect against and/or control a chronic infection when an optimal memory T cell response is generated. The current challenge now is to better define the specific properties of the cellular response that resulted in protection. As a first step toward this goal, the constitutive and poised transcriptional regulatory programs for homing molecules must be assessed in memory T cell subsets from model systems that generate functional memory T cell subsets. Yellow fever and smallpox vaccines are considered model vaccination programs for the successful generation of life-long immunity and memory T cell differentiation. As such, several labs have begun to perform longitudinal phenotypic and functional characterization of the antigen-specific T cell responses generated from these live-viral vaccines [24–26]. In both of these live-viral vaccine systems, a robust effector CD8 T cell response is followed by the persistence of a pool of long-lived memory CD8 T cells. Thus these model systems may be ideal for generation of transcriptional programming data sets of antigen-specific memory T cells that can serve as a reference for evaluating T cells from failed and ongoing vaccine trials.

Epigenetic profiling of T-cell memory differentiation

It is now apparent from both human and nonhuman primate vaccine trials that our limited understanding of HIV-mediated changes to transcriptional regulation in virus-specific CD8 T cells is a significant impediment towards the development of a protective vaccine or functional cure. Thus, model systems that generate functional memory T cells must be utilized to gain a broader understanding of the acquired transcriptional programming that occurs during memory differentiation and the program that is unique to the individual memory subsets.

Tissue and cell-specific transcriptional regulatory programs are acquired during various stages of cellular differentiation and must persist into the cellular progeny to maintain the specialized functions of the cells that comprise a particular tissue. Covalent modifications to histones and DNA, referred to as epigenetic modifications, are utilized to regulate transcription factor and polymerase access to transcriptional regulatory elements in chromatin. Epigenetic modification of chromatin provides cells with a mechanism to retain acquired transcriptional regulation throughout cell division [27]. Given the fundamental role that epigenetic programs play in providing a cellular ‘identity’, tremendous effort has gone into developing methods that can measure these modifications at single genes, and more recently at a whole genome scale.

Affinity enrichment methods relying on antibodies to particular epigenetic marks are commonly used for detecting changes in histone modifications and DNA methylation between two cell types, or different stages of differentiation of a single cell type. The resolution for such techniques is usually restricted to several hundred base pairs. Alternatively, bisulfite-sequencing measurements of DNA methylation is routinely

employed for studies that require nucleotide resolution (Figure 1b). Both methods have been coupled to deep-sequencing technologies to provide whole genome analyses of histone and DNA modifications. In-depth discussion on the resolution, coverage, cost, and material requirements of different strategies for whole genome epigenetic profiling can be found in the following citations [28–31].

Until recently, bisulfite sequencing, often regarded as the gold standard for DNA methylation analysis, has been used to interrogate single or multiple gene regulatory elements (Figure 1b). This method relies on the protection of methylated cytosines from deamination during bisulfite treatment, and subsequent measurement of retained cytosines versus those converted to thymines following PCR amplification and sequencing (Figure 1b, left branch). We have recently used this technique to assess the transcriptional programming of the locus that encodes the PD-1 inhibitory receptor in virus-specific CD8 T cells from yellow fever vaccinated humans [32•]. Yellow fever-specific CD8 T cells underwent demethylation at the PD-1 promoter at the effector stage of differentiation, but to our surprise, the PD-1 regulatory region regained methylation in the yellow fever-specific memory CD8 T cells after viral clearance. Thus, functional memory CD8 T cells retain a mechanism for acquiring new, or modifying existing epigenetic programs. Using a murine model system to further explore this mechanism, we observed that the ability to reacquire the DNA methylation program was lost following prolonged exposure to the antigen during chronic infection [32•]. Consequently, retention of the unmethylated program at the PD-1 promoter in the exhausted CD8 T cells resulted in premature re-expression of the inhibitory receptor. These investigations are currently being expanded to analysis of HIV-specific CD8 T cells from progressors and elite controllers to better understand the role of prolonged antigen exposure on CD8 T cell differentiation during HIV infection (Youngblood *et al.*, submitted for publication). Together, these studies broadly demonstrate that the acclimatization of antigen-specific T cells to the antigenic and inflammatory milieu is coupled to acquired epigenetic programs. Furthermore, these studies illustrate the value of measuring epigenetic programs in resting cells, specifically for analysis of down-regulated genes that are poised for rapid expression. Importantly, the above studies demonstrate that DNA methylation profiling of vaccine generated memory T cells can be performed on a limited number of cells (1000–2000 cells for the above described data sets, although other labs have reported bisulfite sequencing methylation analyses using single cells).

In addition to assessing the role of epigenetic mechanisms in the linear differentiation of antigen-specific CD8 T cells responding to acute versus chronic infections, we have also recently interrogated the dichotomy of CD4 Th1 and Tfh memory differentiation using the LCMV mouse model system of acute viral infection. We observed that granzyme B expression by virus-specific CD4 T cells was restricted to Th1 and not Tfh effector cells. To address the question of lineage commitment at the memory stage of the response, we examined the epigenetic program at the granzyme B locus. Antigen-specific Tfh effector cells maintained a repressive DNA methylation program at the granzyme B locus, while effector Th1 cells had undergone demethylation relative to naïve cells. Importantly, the Th1 and Tfh effector cell epigenetic programs at the granzyme B locus were maintained into the memory stage of the response in the respective Th1 and Tfh cell subsets. Consistent with the differential transcriptional programming at the granzyme B promoter, secondary Th1 effectors cells exhibited a heightened ability to re-express granzyme B, while secondary Tfh effector cells were restricted in granzyme B expression following re-exposure to virus [33]. These data provide a clear example of how analysis of epigenetic modifications at the locus of a lineage-restricted effector molecule will enhance the evaluation of the recall function of vaccine-generated CD4 memory T cell subsets, and specifically highlight analysis of the granzyme b locus as an important metric to be included in epigenetic profiling of vaccine responses.

To attain a broader understanding of the epigenetic programs acquired during T cell differentiation, investigators are now utilizing techniques that provide genome-wide analyses. Initial genome-wide ChIP-seq analysis of memory CD8 T cells were performed measuring histone methylation profiles of human polyclonal memory CD8 T cell subsets [34]. This primary study reported that bivalent histone modifications, a poised epigenetic program, was present upstream of the transcriptional start site of many genes in memory T cells. Consistent with the presence of the poised epigenetic program, transcription from several of these genes was rapidly induced upon anti-CD3 anti-CD28 stimulation. More recent studies using genome-wide ChIP-seq (chromatin immunoprecipitation-sequencing) as well as DNase-seq (DNase digestion of chromatin-sequencing) techniques have evaluated the distribution of permissive and repressive histone modifications that are coupled to Th1-specific and Tfh-specific gene expression programs [35,36•]. These data highlight that permissive histone modifications at loci that encode lineage-regulating transcription factors (Tbx21, Gata3, and Rorc) are predictive of the relative plasticity of Tfh effector cells to become reprogrammed following *in vitro* restimulation under polarizing conditions to other cell lineages [36•]. In another recent study, whole genome MeDIP-seq analysis (Figure 1c) was utilized to study the relationship between DNA methylation and regulatory T cell (Treg) lineages. Data from this study demonstrated that the lineage-defining transcription factor Foxp3 was not sufficient for optimal Treg differentiation and suppressive function without TCR-induced DNA demethylation at genes that are essential for Treg function [37•]. This study highlights that while lineage ‘master regulator’ factors are often used to identify T cell subsets and are required for their differentiation, they are not sufficient for the full development of that lineage when repressive epigenetic modifications to histone and/or DNA prevent the gene expression program of that lineage. Further application of locus-specific and genome-wide epigenetic analyses of antigen-specific CD4 effector and memory T cells will be useful for defining their differentiation state, their relative lineage commitment versus plasticity/pluripotency, and the extent to which gene expression programs remain poised in memory T cells following prime and boost immunization.

Summary

The stage is set for identifying the acquired epigenetic programs unique to individual effector and memory T cell subsets. Recent studies have demonstrated that DNA methylation and histone modification profiling can be used to describe the differentiation state of T cells. Although significant advances in sequencing technologies have reduced the cost and improved upon the resolution of whole genome epigenetic profiling, it may still be impractical to perform such analysis on memory T cells from individual vaccines. Therefore, reference data sets will have to be generated using the above described small animal and human experimental model systems of memory T cell differentiation. From these data sets, a collection of reference gene regulatory programs may be generated that are specific to the different subsets of memory T cells. It will then be possible to use locus-specific PCR based quantification of the memory-subset epigenetic programs as a novel strategy for assessing the quality of vaccine-induced memory T cells.

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Figure 1.

Epigenetic profiling to assess resting memory T cell recall potential. **(a)** Resting memory T cells have acquired transcriptional regulatory programs at genes that code for effector molecules and various receptors. Heightened transcriptional activation can be achieved by acquiring a poised epigenetic program (green DNA), whereas genes that must remain repressed can acquire epigenetic programs that restrict access of transcription factors and polymerase (red DNA with repressive chromatin modifications represented as black lollipops). **(b)** Memory T cell subset delineation is based on the expression of selectins, chemokine receptors, and adhesion molecules. Epigenetic profiling will provide indirect assessment of constitutive or poised tissue-homing and effector molecule expression. **(c)** Potential experimental or clinical workflow for loci-specific or genome-wide DNA methylation analysis of vaccine-generated memory T cells. Following vaccination or infection, genomic DNA is isolated from FACS purified antigen-specific T cells. For single locus analysis (left branch of the flow chart) the genomic DNA is treated with sodium bisulfite to induce deamination of unmethylated cytosines. PCR primers specific to bisulfite converted DNA is used to amplify known transcriptional regulatory regions. The methylation frequency of specific cytosines is calculated as a fraction of the total sequenced cytosines. Whole genome methylation (Right branch of the flow chart) is achieved by coupling MeDIP (methyl-DNA immunoprecipitation) with deep sequencing analysis. Sheared genomic DNA is immunoprecipitated with a methyl-specific antibody. Flow-cell clusters are generated from libraries of the enriched methylated DNA versus total input DNA. Sequencing and quantification of the total reads is performed. Methylated regions are identified as the enriched reads over the total input reads. In the given example, four clonal clusters of the MeDIP-enriched methylated region are present on the platform, while this region is 'equivalently' represented compared to unmethylated DNA sequences from the total input.