



Genetic footprints of T cell exhaustion

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In a recent publication in *Science*, Haining and colleagues describe for the first time the epigenetic landscape of exhausted CD8⁺ T cells in chronic viral infections (1). The results shed some light on how exhausted T cells could be “epigenetically” engineered in chronic viral or cancer immunotherapies.

The constant antigenic burden during chronic viral infections and cancer cause CD8⁺ T cells to develop a state of dysfunction often referred to as T cell exhaustion, characterized by a loss of effector functions and up-regulation of multiple inhibitory receptors (2-6). The result is a population of antigen-specific CD8⁺ T cells that are unable to successfully clear the infection or tumor. However, exhausted CD8⁺ T cells (T_{EX}) do retain some functionality (7,8). Blockade of inhibitory receptors such as programmed death 1 (PD-1), cytotoxic T-lymphocyte associated protein 4 (CTLA-4), Tim-3, and T-cell immunoreceptor with Ig and ITIM domains (TIGIT) has been used as a means of reinvigorating T_{EX} in order to combat cancer and viral infections *in vitro* and in clinical studies [reviewed in (5,9)]. In mice, the use of anti-PD-L1 blocking antibodies was first proven to restore CD8⁺ T cell function against multiple epitopes during chronic lymphocytic choriomeningitis virus (LCMV) clone 13 infection (10). Clinical studies with therapeutic blockade of inhibitory receptor pathways (e.g., PD-1/PD-L1) in the field of human cancer research have rapidly resulted in several drugs approved by the FDA. Although great advances have been made, still, all patients do not respond equally to inhibitory receptor blockade therapy and the durability of the responses differ (5,9). Engineered T cells, such as T cells expressing chimeric antigen receptors (CARs), are another approach to combat malignancies (11,12). However, CAR-T cells also develop exhaustion and require inhibitory receptor blockade in

order to regain their function (13,14).

Instead of PD-1/PD-L1 pathway blockade, PD-1 expression could be decreased by gene editing, made possible by the CRISPR-Cas9 system. A recent study demonstrated a decreased PD-1 expression on primary human cells, using CRISPR-Cas9 (15), suggesting a way to create CAR-T cells with a higher resistance to exhaustion. However, as upregulation of inhibitory receptors represents a way for the immune system to limit the immunopathology caused by prolonged antigen exposure, a more tunable way to prevent exhaustion may be preferential. Recently, such a method was presented, as enhancer-specific editing was demonstrated in human cells (16). Therefore, identifying such regions for *Pdcd1* would be of interest to prevent PD-1 expression, and eventually other inhibitory receptors. However, this requires a deeper understanding of the epigenetic landscape of T cell exhaustion. Identifying regions accessible to transcription factors in T_{EX} as compared to functional T effector and memory cells would provide a picture of the epigenetic landscape affecting T cell exhaustion. This would create the means to edit gene expression to engineer T cells resistant to exhaustion.

Haining and colleagues have thus studied the epigenome of memory and exhausted CD8⁺ T cells in mice and humans (1). They use a newly described assay known as transposase-accessible chromatin with high throughput sequencing (ATAC-seq) that maps chromatin accessibility in the entire genome (17). This methodology uses a hyperactive Tn5 transposase that incorporates adapters into chromatin accessible regions to trace open chromatin. Sequencing reads for specific genome sites are then inferred as a region of increased chromatin accessibility. Using ATAC-seq, researchers can more easily than previously

conduct genome-wide studies of open chromatin sites, nucleosome positioning and transcription factor footprints. ATAC-seq is less time consuming and requires fewer cells than other conventional methodologies in the field, making it suitable of studying chromatin accessibility in rare populations such as antigen-specific T cells or tumor infiltrating lymphocytes (TILs).

Haining and colleagues demonstrate that both differentiation and exhaustion of CD8⁺ T cells is associated with extensive chromatin reorganization during infection with acute (Armstrong) and chronic (clone-13) LCMV infection (1). In fact, CD8⁺ T cell differentiation and exhaustion is accompanied by more extensive chromatin remodeling than those changes seen for gene expression. Through clustering analysis, specific modules of chromatin accessible regions were identified that positively correlated with the average gene expression of the adjacent genes. These data indicate that open chromatin sites adjacent to specific genes represent activating, rather than repressing, regulatory elements for different subsets of CD8⁺ T cells. The authors further probed in on the *Pdcd1* gene locus to define unique regulatory regions specific to T_{EX}. They identify nine chromatin accessible regions (ChARs) adjacent to the PD-1 locus present in both acute and chronic LCMV infection and a novel ChAR (–23.8 kb) found only in T_{EX} from chronic LCMV infection. They further prove that the –23.8 kb ChAR functions as an enhancer of PD-1, using CRISPR-Cas9 nuclease deletion in the murine T cell line EL4, constitutively expressing high levels of PD-1. Using transcription factor footprint analysis, it was confirmed that specific transcription factors (such as RAR, Sox3 and T-bet) had an enriched binding motif to the –23.8 kb region and exhausted CD8⁺ T cells in general.

In a set of experiments conducted in human exhausted HIV-specific CD8⁺ T cells (from four HIV-1 infected individuals), Haining and colleagues show that a majority of the ChARs found in the mouse model have human orthologous regions. Thus, the epigenetic profiles of T_{EX} were in most cases conserved between mouse and man. However, this was not true for the *Pdcd1* gene locus, why a human ortholog for the –23.8 kb enhancer was not identified. Future studies are needed to verify ChARs for the *Pdcd1* gene locus in human antigen-specific cells in blood, different tissues, and TILs. Finally, Haining and colleagues demonstrate in a chronically HCV infected individual that CD8⁺ T cells specific for a conserved HCV-epitope (C63B) displayed an exhausted phenotype and showed greater chromatin accessibility at exhaustion-specific regions. In the same individual, CD8⁺ T cells targeting a variable HCV-epitope (C63B) did not display an exhausted phenotype and showed greater chromatin accessibility in memory-specific regions, illustrating that the

chromatin accessible regions are highly dependent on the level of exhaustion of specific CD8⁺ T cells.

Although therapeutic blockade of the PD-1 pathway shows beneficial outcome in treating several cancer types (5), resistance and relapses are common. Resistance is linked to T cell exhaustion, upregulation of inhibitory receptor ligands on the tumor cells, and interferon signaling allowing for STAT1-related epigenomic changes (18). Haining and colleagues show that exhausted mouse and human CD8⁺ T cells display a specific accessible chromatin landscape with enhancer regions containing enriched binding motifs for specific transcription factors. In the same issue of *Science*, Wherry and colleagues show that exhausted CD8⁺ T cells reinvigorated during PD-L1 blockade have an epigenetic profile distinct from memory T cells (19). This epigenetic state is maintained after PD-1/PD-L1 blockade. Similarly, the state of T cell exhaustion is maintained after adoptive transfer to an antigen free environment or in successfully treated HIV-infected individuals (6,7,20). Thus, distinct epigenetic profiles have a clear impact on the function and phenotypes of CD8⁺ T cells and their ability to clear viral infection or cancer. Current immunotherapies might therefore have limited ability of success if the epigenetic barrier of T cell exhaustion is not reverted or bypassed; especially if the targeted viral or cancer antigen persists after therapy. Different strategies need to be tested to improve future treatment options. It may be possible to selectively prime or improve the functional and phenotypic state of the T cells during blockade of inhibitory receptor pathways (19,21–23). Potentially, epigenetic modification of CAR-T cells might render them resistant to T cell exhaustion (15,16,24). Further investigations will be needed to maximize the benefit of future T cell-based immunotherapies to treat chronic viral infections and additional cancer types.

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