Review

Tolerance and exhaustion: defining mechanisms of T cell dysfunction

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CD8 T cell activation and differentiation are tightly controlled, and dependent on the context in which naïve T cells encounter antigen, can either result in functional memory or T cell dysfunction, including exhaustion, tolerance, anergy, or senescence. With the identification of phenotypic and functional traits shared in different settings of T cell dysfunction, distinctions between such dysfunctional states have become blurred. Here, we discuss distinct states of CD8 T cell dysfunction, with an emphasis on: (i) T cell tolerance to self-antigens (self-tolerance); (ii) T cell exhaustion during chronic infections; and (iii) tumor-induced T cell dysfunction. We highlight recent findings on cellular and molecular characteristics defining these states, cell-intrinsic regulatory mechanisms that induce and maintain them, and strategies that can lead to their reversal.

T cell activation and differentiation can result in functional memory or T cell dysfunction

When naïve CD8 T cells encounter (foreign) antigen in a stimulatory and inflammatory context (e.g., acute infection), a cell-intrinsic program is initiated that drives responding CD8 T cells to expand greatly and differentiate into cytotoxic effector cells that control and eventually clear the pathogen/antigen (expansion phase). Effector T cells secrete high amounts of effector cytokines [e.g., interferon (IFN)γ and tumor necrosis factor (TNF)α], and produce cytolytic molecules (e.g., granzymes and perforin). After the peak of the response, if the pathogen/antigen has been eliminated, most effector T cells undergo apoptosis (contraction phase), but a fraction survive and differentiate into central memory and effector memory T cells (memory phase) (Figure 1). Genome-wide molecular profiling has revealed that naïve, effector, and memory T cell differentiation states each have unique gene signatures that dictate their functional and phenotypic properties [1,2].

CD8 T cell differentiation is tightly controlled, and changes in the nature, context, and duration of antigen encounter can cause substantial alterations in the T cell activation and differentiation process; potentially leading to T cell dysfunction, unresponsiveness, and/or even death. Various states of T cell dysfunction have been described as a consequence of altered activation and differentiation processes, and, depending on the experimental or clinical settings and phenotypic and functional features of the T cells, terms such as exhaustion, tolerance, anergy, senescence, and even ignorance have been used to describe the dysfunctional state (Table 1).

A large number of inhibitory receptors associated with dysfunction have been identified, with most characterized and functionally assessed in a mouse model of T cell exhaustion during chronic viral infection [3,4]. Subsequently, most of these receptors have also been detected on T cells in different experimental and clinical settings of T cell dysfunction, including tumor-reactive T cells in cancers, self-tolerant T cells, and exhausted T cells in the context of other mouse and human chronic infections [5–9]. With the identification of phenotypic traits shared in different settings of T cell dysfunction, distinctions between such states have become blurred, resulting in confused use in the literature of the words exhaustion, tolerance, anergy, and ignorance. Clear definitions for such terms based on their functional traits and molecular choreography are needed to facilitate interpretation of basic and clinical research findings and selection of strategies to modulate T cell dysfunction in different settings.

Here, we discuss the various states of T cell dysfunction, focusing on two well characterized and defined settings: peripheral CD8 T cell tolerance to self-antigens (self-tolerance) and CD8 T cell exhaustion during chronic infections – disparate settings that have in common the persistence of the inciting antigen. We highlight recent findings on the cellular and molecular characteristics that define these two states, the cell-intrinsic regulatory mechanisms that induce, mediate, and maintain them, and strategies and factors that can lead to their reversal. As tumor-reactive CD8 T cells in the context of established cancers can feature similar characteristics as exhausted virus-specific CD8 T cells during chronic infection, aspects of tumor-induced T cell dysfunction are also discussed.

Induction and characteristics of self-tolerance

Tolerance in self-antigen-specific T cells is a dysfunctional state required to prevent autoimmunity (self-tolerance). Unresponsiveness to self results from both central and

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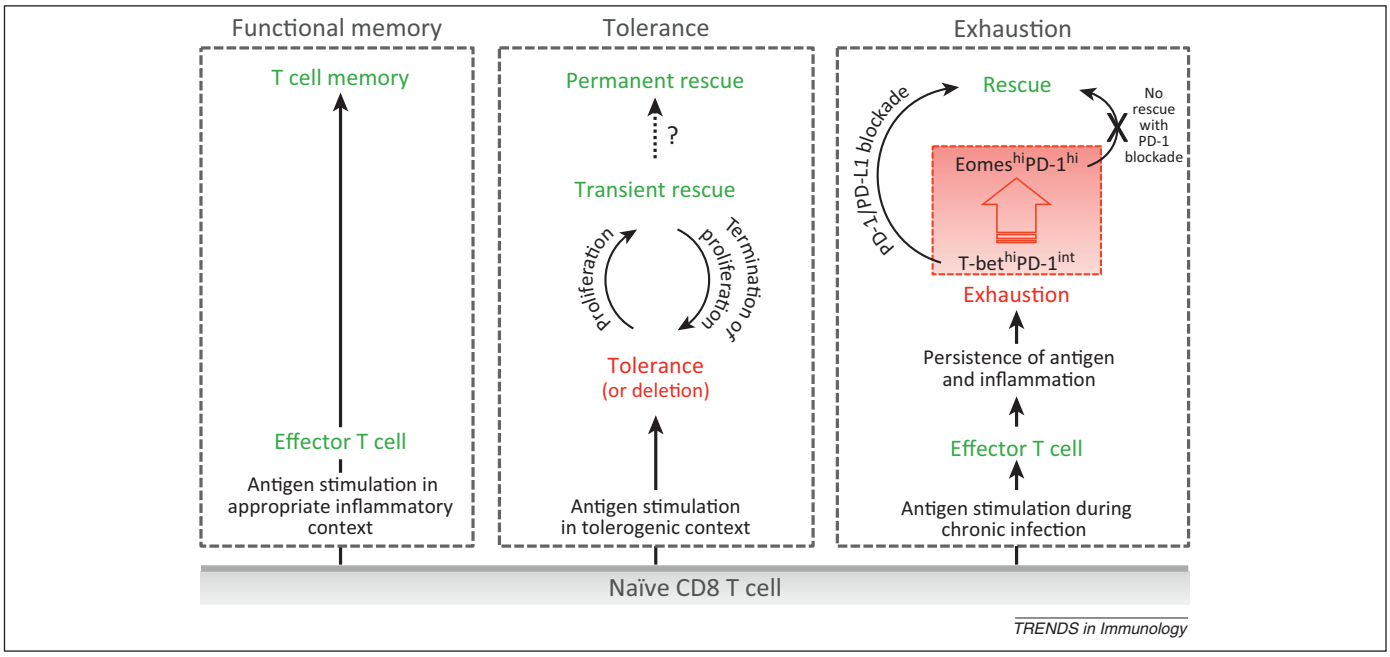

Figure 1. T cell differentiation of naïve CD8 T cells results in either functional T cell memory or T cell dysfunction as reflected by self-tolerance or exhaustion in chronic infections. Functional memory: when naïve CD8 T cells encounter (foreign) antigen in a stimulatory and inflammatory context (e.g., acute infection), T cells differentiate into effector and eventually into memory T cells. Tolerance: peripheral self-reactive CD8 T cells that encounter self-antigen in a tolerogenic context acquire a program of functional unresponsiveness. Tolerant T cells can be transiently rescued by inducing cell proliferation, for example, by cytokines [interleukin (IL)-2 and IL-15] or lymphopenia. However, once proliferation stops, rescued self-reactive T cells are retolerized. If self-tolerant T cells can be permanently reprogrammed and rescued remains to be determined. Exhaustion: virus-specific T cells initially acquire some effector functions early during chronic infections, but, due to persistence of viral antigen and inflammation, T cells become progressively exhausted. Exhausted T cells represent a heterogeneous T cell population containing T-bet^{hi}PD-1^{int} and Eomes^{hi}PD-1^{hi} subpopulations (see text). T-bet^{hi}PD-1^{int} but not Eomes^{hi}PD-1^{hi} exhausted T cells can be functionally rescued by PD-1 blockade.

peripheral immune tolerance mechanisms (Table 1). Central tolerance is established during T cell development in the thymus, with thymocytes expressing T cell receptors (TCRs) of too high affinity for self-antigen/MHC complexes eliminated (negative selection) [10]. However, central tolerance is incomplete, in part because not all peripheral self-antigens are adequately presented in the thymus; self-reactive T cells that escape negative selection must be inactivated in the periphery by a series of tolerizing mechanisms that can include deletion [11–13], suppression by regulatory CD4 T cells [14], and/or induction of cell-intrinsic programs that force self-reactive T cells into a state of functional unresponsiveness [9,15,16]. T cell fate following peripheral encounter with self-antigen is partly dictated by the activation state of the antigen-presenting cell (APC) [17,18]: T cells encountering self-antigen presented by nonactivated or nonprofessional APCs receive incomplete priming signals, and either undergo programmed cell death or become functionally tolerant, exhibiting an antigen-experienced CD44^{hi} phenotype. Such peripheral tolerance is manifested in the inability of tolerant T cells to proliferate and expand in number in response to antigen stimulation, but may not necessarily completely disrupt effector functions such as cytolytic activity and effector cytokine production (split tolerance) [19]. In some settings maintenance of tolerance requires continual exposure of T cells to the self-antigen [20–22], whereas in others the impairment of self-reactive T cells is more profound and even withdrawal of antigen is not adequate to reverse the unresponsive state [9]; likely reflecting differences in antigen level, the nature and site of exposure, and T cell avidity.

Self-tolerance versus self-ignorance

Self-reactive T cells can fail to provoke autoimmune disease due to ignorance (Table 1): when anatomical barriers sequester antigen from immune surveillance (immune privileged site), or when self-antigen is expressed and/or cross-presented at concentrations too low to stimulate T cells, peripheral self-reactive T cells can simply remain 'unaware' or 'ignorant' of self-antigen [23–27]. Thus, 'self-ignorant' T cells, in contrast to self-tolerant T cells, are not rendered dysfunctional from self-antigen encounter, but are antigen-inexperienced and persist as naïve, potentially functional T cells in the periphery. If self-ignorant T cells become activated by external stimuli (e.g., by infection [24,28], inflammatory stimuli [29,30], or cytokines [31]), ignorance can be easily overcome, potentially inducing autoimmunity.

Self-tolerance – a unique state of T cell differentiation with a tolerance-specific gene program

Genome-wide transcriptional analysis has revealed self-tolerant CD8 T cells to harbor a tolerance-specific gene program, with hundreds of genes differentially expressed compared to their naïve or memory counterparts [9]. Self-tolerance thus represents a distinct state of T cell differentiation. Functional impairment of self-reactive T cells was shown to be associated with: (i) lack of expression of genes encoding effector molecules (*Infγ*, *Prf1*, *Gzmm*, and *Grn)*, and altered expression of master transcription factors (*T-bet*, *Eomes, Gata3*, *Egr1*, and *Egr2)* and chemokine and cytokine receptors (*CXCR3*, *CCR5*, and *IL-12Rβ*); (ii) expression of genes associated with reduced immune function and exhaustion (e.g., *Lag-3*); and (iii) expression of