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Like Parent, Like Child: Inheritance of Effector CD8$^+$ T Cell Traits

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Beuneu et al. (2010) report that the amount of antigenic stimulation initially sensed by naive CD8$^+$ T cells can establish differentiation set points that are stably maintained in clonal progeny to promote functional diversity.

During viral and intracellular bacterial infections, CD8$^+$ T cells undergo profound clonal expansion, often yielding millions of cytotoxic effector T cells (CTLs) that play crucial roles in host immunity. These effector cells show newly acquired functional properties such as the ability to migrate to inflamed tissues, produce cytokines (IFN-γ and TNF-α) and chemokines (RANTES), and kill infected cells by delivering cytotoxic granules containing perforin and granzymes. Although CTLs possess many of these properties in common, it has become clear that even within a single antigen-specific CD8$^+$ T cell population, effector cell differentiation does not occur uniformly and results in substantial heterogeneity in the types of and amounts of the cytokines and chemokines and cytotoxic and costimulatory proteins they express. This heterogeneity is imprinted in the memory cells that descend from these cells after infection, creating numerous subsets of memory T cells that have different modes of homeostasis and roles in immunity against reinfection (Cui and Kaech, 2010; Lanzavecchia and Sallusto, 2002).

Although the heterogeneity of effector CD8$^+$ T cells is evident, it is less clear how this diversity is achieved during immune responses. Prior elegant studies have shown that a single naive T cell can expand and differentiate into a heterogeneous pool of effector and memory CD8$^+$ T cells, indicating that the fate of naive T cells is modulated by the signals encountered during priming (Gerlach et al., 2010; Stemberger et al., 2007). The study by Beuneu et al. (2010) in this issue of *Immunity* focuses in on the "first kiss" between the dendritic cell (DC) and T cell and finds that the strength of antigenic signals can directly affect the amount of IFN-γ, TNF-α, and IL-2 expressed by activated CD8$^+$ T cells, hence, uncovering another critical factor that creates functional diversity in CTLs (Beuneu et al., 2010).

The strength of antigenic stimulation for CD8$^+$ T cell activation is determined by many factors including antigen abundance, duration, and affinity for MHC class I and the TCR. A brief initial DC-T cell encounter can trigger continued proliferation and effector and memory CD8$^+$ T cell development (references within Cui and Kaech, 2010), but prolonged or repetitive DC-T cell interactions or increased antigen abundance or affinity during infection can drive greater CTL differentiation and expansion (Henrickson et al., 2008; Joshi et al., 2007; Sarkar et al., 2008; Wherry et al., 1999; and references within Cui and Kaech, 2010; Lanzavecchia and Sallusto, 2002). More intense TCR activation can also hasten DC-T cell conjugation and induce the development of more "fit" effector T cells that respond well to memory T cell homeostatic cytokines IL-7 or IL-15 (Henrickson et al., 2008; Lanzavecchia and Sallusto, 2002). Signals emanating from costimulatory receptors and inflammatory cytokines are also critical for amplifying the strength of signal and effector CD8$^+$ T cell differentiation. Based on these findings, a progressive model of T cell differentiation has been proposed during an immune response wherein at one extreme end of a spectrum lie anergic T cells, formed by insufficient T cell stimulation, and at the other end lie terminally differentiated effector cells prone to death from excessive stimulation. In between these ends lie effector T cells that have differentiated in a graded manner according to their collective history of stimulation, of which some become most fit to persist as memory T cells (Cui and Kaech, 2010; Lanzavecchia and Sallusto, 2002).

The current study focused on how the strength of antigenic signal during the initial period of T cell activation influenced the functional diversity in CTLs, namely their ability to produce IFN-γ. The authors immunized mice containing OVA-specific OT-1 CD8$^+$ T cells, which expressed an IFN-γ reporter transgene, with DCs pulsed with varying doses of antigen, and they tracked the progeny of individual activated CD8$^+$ T cells in vitro and in vivo via intravital microscopy and flow cytometry. Although the dose of peptide in this study affected neither the duration of DC-T cell contact nor the expansion of T cells, higher antigen doses induced greater numbers of IFN-γ-producing CD8$^+$ T cells and amounts of IFN-γ produced on a per cell basis in comparison to lower antigen doses (Figure 1). Moreover, stronger antigenic signaling also augmented the formation of "polyfunctional" effector CD8$^+$ T cells that can coproduce IFN-γ, TNF-α, and IL-2. Although this aspect was not probed further in this study, polyfunctional CD8$^+$ T cells have been associated with greater memory cell potential, proliferative responses to IL-15, -7, and antigen, and protection after vaccination (Joshi et al., 2007; and references within Cui and Kaech, 2010). Interestingly, the authors could "see" the differential expression of IFN-γ in the activated CD8$^+$ T cells within the first 24–40 hr of activation, before

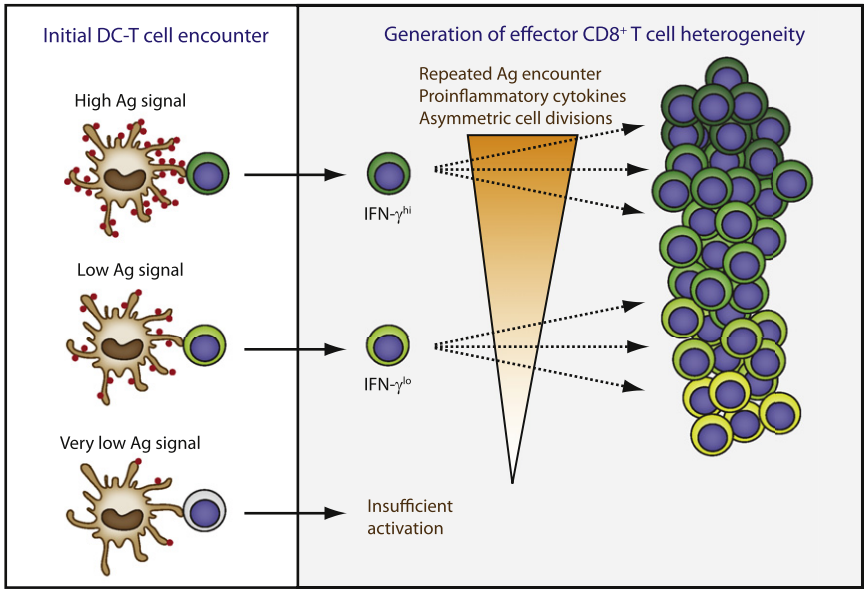

Figure 1. Model for Generating Functionally Diverse Effector CD8$^+$ T Cell Populations
When naive CD8$^+$ T cells encounter antigen-presenting DCs, the initial strength of antigenic signal can establish thresholds of effector cell differentiation (such as the amount of IFN-γ expression), which can be stably inherited in clonal progeny without further stimulation. However, additional factors (at the time of priming or throughout the response) such as repetitive Ag encounter, inflammation, costimulation, and asymmetric cell divisions may further modify the differentiation state of the effector CD8$^+$ T cell in a progressive manner, heightening the functional and phenotypic heterogenity of antigen-specific CD8$^+$ T cell populations.

commencement of the first cell division. These findings suggested that the strength of antigenic signal first encountered by naive T cells on DCs could be an early determinant of effector function and differentiation, but because antigen abundance was the only parameter varied in these studies, it will be important to see how altering other parameters, such as costimulation, antigen affinity, or inflammation, in the presence of lower antigen doses affect the overall signal strength and differentiation set points of newly activated CD8$^+$ T cells.

The authors went on to demonstrate that the strength of initial DC-T cell contact could seemingly set a "threshold" of effector CD8$^+$ T cell differentiation that was stably maintained in the clonal progeny of the originally activated CD8$^+$ T cells. OT-1 T cells were primed in vitro or in vivo for 24–40 hr and then individually cloned and grown in culture for a few more rounds of division. Interestingly, "like parent, like child," the amount of IFN-γ production first observed in the parental CD8$^+$ T cell was stably maintained in its daughter cells. Thus, in the context of DC immunizations, the initial strength of antigenic signal could modulate the extent of CTL differentiation in a manner that was stably inherited over space and time to generate functionally distinct pools of effector CD8$^+$ T cells. However, this study did not analyze how the maintenance of such differentiation set points were affected by repetitive stimulation or inflammation—factors that would normally be encountered in the settings of infection, vaccination with attenuated live pathogens, or tumor eradication and would contribute to the functional heterogeneity of effector CD8$^+$ T cells. Indeed, greater amounts of inflammatory cytokines, such as IL-12, IFN-γ, and IL-2, and longer durations of stimulation or infection enhance terminal effector CD8$^+$ T cell formation (Joshi et al., 2007; Sarkar et al., 2008; and references within Cui and Kaech, 2010; Lanzavecchia and Sallusto, 2002). It may be possible, without further stimuli, for a briefly stimulated CD8$^+$ T cell to stably pass on its differentiation set-point to its progeny relatively uniformly; however, lineage tracing of effector CD8$^+$ T cells have shown that a fairly heterogenous effector cell pool can arise from a single naive antigen-specific CD8$^+$ T cell during infection (Gerlach et al., 2010; Stemberger et al., 2007). Furthermore, the ability of activated CD8$^+$ T cells to divide in an asymmetric manner provides a mechanism for establishing diversity in effector functions and cell fates within the first cell division during infection (Chang et al., 2007). The ability of T cells to divide asymmetrically is context dependent and was observed when CD8$^+$ T cells were activated during infection, but not when the immunological synapse was compromised (in infected ICAM1-deficient animals) or when T cells divided under lymphopenic conditions (Chang et al., 2007). Thus, it is possible that the DC priming conditions used in the current study did not elicit asymmetric cell divisions, which contributed to the functional uniformity of the daughter cells that descended from the parental activated CD8$^+$ T cell. Altogether, these studies suggest that the amount of antigenic stimulation alone could be a dominant factor in establishing functional heterogeneity within CD8$^+$ T cells, but normally additional differentiation cues, present during priming or possibly even later, can be integrated to create diverse effector pools (Figure 1). This point is strengthened by the observation that despite variations in immunodominance during acute viral infections, different epitope-specific CD8$^+$ T cell populations often share considerable, but not identical, overlap in their phenotypic and functional heterogeneity. This work highlights that the strength of antigenic stimulation can be instructive in forming diverse and polyfunctional effector cells and may be most relevant in cases of nonreplicating vaccines where inflammation as well as antigen dose and duration are limited. It will be important to better understand how antigen doses influence memory T cell diversity and protective immunity to aid in vaccine design.

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Even Neurons Are Excited by Th17 Cells

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The mechanisms by which T helper 17 (Th17) cells contribute to autoimmune encephalomyelitis are likely diverse and not fully elucidated. In this issue of *Immunity*, Siffrin et al. (2010) propose that Th17 cells engage in direct interactions with neurons, leading to neuronal dysfunction and exacerbation of disease.

Multiple sclerosis (MS) is an autoimmune disease in which an inappropriate immune response to central nervous system (CNS) antigens leads to demyelination and, ultimately, axonal injury. Experimental autoimmune encephalomyelitis (EAE), a common animal model for MS, has been instrumental for dissecting the contributions of various hematopoietic and nonhematopoietic cell types to the pathogenesis of this disease. Over the past decade, multiple lines of evidence have demonstrated a critical role for T helper 17 (Th17) effector T cell responses and, in particular, their effector gene product, IL-17A, in the development of EAE (Komiyama et al., 2006). Although it is surely more complex in its pathophysiology than these EAE models, emerging evidence suggests that Th17 responses may also underlie critical aspects of MS in humans, thus emphasizing the importance of elucidating the function of these cells during CNS inflammation (Kebir et al., 2007).

The mechanisms by which Th17 cells contribute to autoimmunity, and MS in particular, have been the subject of intensive study. Although the precise role of these cells in EAE remains controversial, several recent studies have begun to define the Th17 cell-related cellular and molecular pathways involved in disease pathogenesis. Following activation and differentiation in secondary lymphoid organs, Th17 cells expressing the chemokine receptor, CCR6, enter systemic circulation and appear to invade the CNS through epithelial cells of the choroid plexus, which express the CCR6 ligand, CCL20 (Reboldi et al., 2009). Once localized within the CNS, these autoreactive Th17 cells presumably interact with antigen-presenting cells (APCs) presenting myelin-derived antigens, leading to production of effector cytokines, such as IL-17A. In addition to Th17 cells, other cell types, including $\gamma\delta$ T cells, are potential sources of IL-17 in the CNS (Sutton et al., 2009). Several studies propose that IL-17A mediates the major pathogenic functions of Th17 in EAE, supporting a model in which the local production of IL-17A leads to activation of endothelial cells within the CNS, thereby disrupting the blood brain barrier (Kebir et al., 2007), and upregulation of proinflammatory chemokines and cytokines from CNS-resident astrocytes or other cell types that are capable of amplifying the autoimmune response by recruiting additional immune cell populations (Kang et al., 2010; Ogura et al., 2008). Thus, in this scenario, Th17 cells serve to initiate and potentially sustain the recruitment of a secondary pathogenic inflammatory cell infiltrate that is directly responsible for the demyelination and neuronal damage underlying the physiological manifestations of the disease (Figure 1).

The above model proposes a role for Th17 cells in EAE that is in line with the function of these cells during immune responses to extracellular pathogens, i.e., the IL-17-dependent recruitment of immune cell types, such as neutrophils, that directly eradicate the infectious agent. However, Th17 cells likely have additional unexpected roles in infection and autoimmunity beyond these relatively simplistic models. Indeed, studies have indicated that IL-17A and IL-17F may not be the major pathogenic cytokines in certain EAE models, suggesting the existence of additional mechanisms by which Th17 cells can modulate disease (Haak et al., 2009).

In this issue of *Immunity*, Siffrin et al., based on their intravital imaging observations of Th17 cell behavior during EAE, propose an intriguing model of Th17 cell-mediated pathogenesis involving direct, antigen-independent T cell-neuron interactions (Figure 1). Although several studies have examined T cell dynamics during EAE using intravital microscopy