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Lineage relationship of effector and memory T cells

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Abstract

Adaptive immunity is characterized by the ability to form long-lived immunological memory. Upon re-exposure to antigen, memory T cells respond more rapidly and robustly than naïve T cells, providing better clearance of pathogens. Recent reviews have reinforced the text-book view that memory T cells arise from effector cells. Although this notion is teleologically appealing, emerging data is more consistent with a model where naïve cells directly develop into memory cells without transitioning through an effector stage. A clear understanding of the lineage relationships between memory and effector cells has profound implications for the design of vaccines and for the development of effective T cell-based therapies.

Introduction

Immunological memory is the key distinguishing hallmark of the adaptive immune system [1]. Through their expression of massively diverse receptors, T cells are capable of exquisite specificity. The rearrangement of the genes encoding these T cell receptors (TCR) occurs in the thymus, which generates ‘naïve’ cells endowed with considerable epigenetic plasticity. Following antigenic stimulation, naïve $CD8^+$ T cells can differentiate into ‘effector’ cells that produce inflammatory cytokines and cytotoxic molecules and into ‘memory’ cells, which are capable of an enhanced response to subsequent encounters with their cognate antigen. The widely held concept that effector T cells give rise to memory cells [2,3] has a certain intuitive and teleological appeal because memory T cells *should* arise from the effector cells that eliminated pathogens after a primary infection. This reasoning is also consistent with the observed natural history of a $CD8^+$ T cell response in which there is a massive expansion of effector cells that is coincident with the elimination of the pathogen and later, over time, there is a ‘transition’ into the predominance of memory cells. It also seems plausible to some that effector cells do not give rise to memory cells but rather represent a terminally differentiated state, ie memory cells come developmentally before effector cells and not *vice versa* [4-7]. This model of differentiation, which has analogies to developmental systems, might involve asymmetric division of progenitor cells [8] and it may result from progressive differentiation of naïve cells into memory cells and ultimately effector cells [5,6].

Roadblocks in the determination of T cell lineage relationships

It is surprising that there continues to be a great deal of debate about the lineage relationship between effector and memory T cells. Despite the importance of understanding these relationships – and a growing body of knowledge of the molecular aspects of T cell immunobiology – there remains a robust debate in the field about the relationships of effector and memory T cells [9,10]. As with many debates, the most forcefully held opinions are sometimes held where the information available is most sparse.

How is it that the question of the developmental biology of post-thymic T cells can be so murky whereas other adult systems are more clearly understood? We feel that a major roadblock in the study of T cell maturation and differentiation is simply the lack of clear anatomical relationships among T cell subsets. In most other biological systems, the developmental biology of cellular constituents can be determined in large part by observing the anatomical locations of the cell experiencing maturation. The location and movement of cells within any given anatomical location can provide clues as to the lineage relationships of cells (Figure 1A and B). Differentiation of cell types from stem cells continues in adult organisms, where histologic structures can provide rich evidence for cellular differentiation pathways.

For example cells of the skin are located in specific anatomic relationships relative to other structures. Skin stem cells reside in a structure called the ‘bulge’ and migrate up and down the hair shaft to refresh new dermal structure [11] (Figure 1B). Cells of the small intestine also have precise anatomical locations in the adult animal. Stem like cells reside deep within the crypts and then they move progressively towards the tips of the villi before they slough off and die [12] (Figure 1A). Post-thymic T cells are motile within the blood and lymphoid tissues, so anatomic clues are not readily apparent. Although intravital microscopy may yield new clues [13], T cells existing at a variety of developmental stages can exist within the same anatomical space (Figure 1C).

In addition to the lack of clear anatomical relationships, other problems have complicated the study of the lineage relationships of T cell subsets. One of the conventional surface proteins used to distinguish memory from effector cells – CD62L – is rapidly cleaved upon T cell activation by a disintegrin metalloprotease called ADAM17 [14-16]. It seems untenable to draw conclusions about effector and memory development based on sorting of $CD62L^+$ and $CD62L^-$ cells [17] because the lack of CD62L does not identify *bona fide* effector cells. Both effector cells as well as recently primed but minimally differentiated T cells lack CD62L on their surfaces: the former cells do not express CD62L transcripts, whereas the later population continue to express this gene product but appear to be negative because CD62L protein is cleaved from the cell surfaces.

Lineage relationships of memory and effector cells have also been complicated by the use of reporter systems that have been seriously flawed. Genetic tagging systems controlled by the granzyme B [18,19] or IFN-γ [20] promoters have been employed to indelibly mark effector T cells and track their fate. These reporter systems however are not designed to measure gradients of expression of the molecule being studied. Reporter systems employed to date are too sensitive and provide binary answers. Thus, genes whose expression is turned on in a progressive manner will be reported as ‘yes’ even if the system is measuring small amounts of the gene in question. Thus, it is likely that these genetic tagging systems identify cells that have been activated rather than marking fully differentiated effector cells.

Two competing models for the relationship of effector and memory T cells

It is manifestly obvious that pathogen-specific T lymphocytes will become effector cells after an infection. It is also clear that pathogen-specific T cells will have a phenotype of memory cells after longer periods of time. However, these irrefutable observations do not necessarily indicate that individual virus-specific effector cells morph into memory cells. The question at hand regards the developmental fates and differentiation of individual T cells and not populations of cells.

The dominant model for $CD8^+$ T cell differentiation is that T cells react to pathogens by experiencing a burst of proliferative activity. During this time, T cells become highly activated and produce copious amounts of effector cytokines (such as IFN-γ, TNF-α and GM-CSF). They also become capable of direct cytolysis and are capable of deploying perforin and granzymes. In this model, most of these cells die, but some of them survive to become memory cells. These memory cells purportedly emerge from the effector population stochastically, or are selected because of their superior ‘fitness’ or avidity for the pathogen-associated antigens. This model (shown in Figure 2, left) has tremendous intuitive and teleological appeal because it predicts that the effector cells that actually are capable of destroying the pathogen give rise to memory cells. Proponents of this theory contend that all memory cells indeed were at one point effector cells that experienced a reversion from an active, lytic state into a state that more closely resembles naïve cells. This model of reversion invokes a pattern of ‘off-on-off’ and ‘on-off-on’ changes in the phenotype, metabolism and gene expression patterns within individual T cells [21].

The alternative to this model has it that memory cells do not arise from effector population, but rather are derived directly from activated naïve cells that never experience a full-blown highly cytotoxic effector state. In this model, rather than differentiating to memory cells, effector cells represent a terminally differentiated state that can only give way to more effectors or to senescent or dead cells. This is model is reminiscent of developmental models that include stem cells (Figure 2, right panel) that maintain themselves while also giving rise to progeny cells that experience progressive differentiation into mature functional tissues.

The models represent polar opposites of T cell development and lead to radically different predictions for the behavior of individual T cells. The debate is not merely one of nomenclature and semantics. The ‘off-on-off’ model predicts that individual T cells become granule-containing, highly cytotoxic effector cells after exposure to antigen, and that after elimination of the antigen most of these cells die but some experience a gradual transition to memory cells, whereas the developmental model would have effector cells dying off and memory cells arising as a separate population of cells that never experiences a full-blown effector state (Figure 2A). In this later model, memory cells are activated, but do not experience full-strength or repeated antigenic stimulation in a highly inflammatory milieu. These conditions exist towards the end of an infectious event, and are consistent with experiments showing that naïve antigen-specific T cells added during the tail-end of an infection are more prone to form memory [22].

The two models also have very different predictions for the replicative capabilities of individual T cells. The off-on-off model predicts that memory cells have proliferated more or the same extent of the effector cells from which they were purportedly derived. This view, however, is at odds with the findings that length of telomeres [23-25] and activity of telomerase [25] are both reduced in effector compared to memory cells. These data are, instead, consistent with the developmental model, which predicts that individual memory cells have proliferated less than the effector cells that will be derived from their progeny (Figure 2B). The predicted multipotency of cells in the off-on-off model is that effector cells

are capable of becoming memory cells [2,17] whereas the developmental model predicts that this effector to memory cell transition does not happen and that effector cells exist in a terminal state that results in cell death but not formation of memory [26] (Figure 2C). Here again, the developmental model is more consistent with varieties of experimental data. Repeated antigenic stimulation of T cells leads to increased effector functions but impaired memory [27,28]. Conversely, memory T cell subsets are demonstrably capable of differentiating into both memory and effector subsets whereas effector cells are incapable of differentiating into memory cells *in vitro* [26].

As its name implies, the off-on-off model would predict that gene expression in individual cells that are turned on upon antigenic stimulation are gradually turned off upon resolution of a pathogenic infection. Of course, this model also implies that genes associated with naïve cells are turned off during infection and turned on again during the gradual reversion of effector cells into memory cells [29] (Figure 2D). The aforesaid also applies to changes in chromatin structure – where T cells experience massive changes to their chromatin which are substantially undone during their hypothesized transition into memory cells [21] (Figure 2E). This 'flip-flopping' of chromatin would represent a significant departure from patterns of progressive chromatin changes that are observed in other developmental systems [30]. By sharp contrast, a model where naïve T cells first transition into memory cells and then transition into effector cells would be more in line with other biological systems [26,31,32].

It seems difficult or impossible to reconcile the notion that there effector to memory transition is correct given that many genetic or environmental stimuli that promote T cell effector differentiation are accompanied by impairment in memory formation and *vice versa* [33-47].

What we have learned from the analysis of individual T cells

Emerging technologies enable the integration of large amounts of new data that influence perceptions of these new models for cellular differentiation. The differentiation of $CD8^+$ T cells has now been studied at the level of individual T cells. Adoptive transfer of T cells individually labeled with heritable congenic markers or DNA 'barcodes' has recently enabled investigators to trace the progeny of single T cells and gain new insights on developmental models of memory generation [48-50]. These studies have revealed an extreme heterogeneity in the behavior of individual naïve cells in response to infection. Specifically, T cells that experienced massive proliferation tend to generate short-lived $KLRG\text{-}1^+$ effector cells, whereas minimally expanded T cells preferentially form long-lived $CD62L^+$, $CD27^+$ cells [48-50]. These findings are consistent with a developmental model where proliferation and differentiation are tightly linked. In this model, naïve cells proliferate to generate memory cells first and then effector cells, a concept we think is further supported by recent findings posthumously published by Leo Lefrançois. His team tracked the fate of individual naïve and memory T cells upon adoptive transfer in the same hosts. As predicted by a model where T cells experience progressive differentiation, the descendants of single memory cells were largely short-lived $KLRG\text{-}1^+$ effector cells, whereas the progeny derived from single naïve CD8+ T cells were preferentially memory cells [50].

These studies using single cell analysis together with T cell subpopulation studies previously published by our group and others all point to the same conclusion. Like other developmental systems in adult organisms that employ stem cells, $CD8^+$ T cell differentiation is largely a linear and unidirectional process [5,6,51] (Fig 3). This process is associated with characteristic changes in cell surface molecules and a shift in metabolism from one based on lipid oxidation to one based on glycolysis [52-54] (Fig 3). In this model,

T cell receptor engagement in the context of an inflammatory milieu stimulates T cells to proceed on a pathway of differentiation. In this model supported by a wealth of evidence, it is the strength of the signal that determines how much differentiation T cells experience. The full panoply of inputs that T cells integrate to embark on differentiation is complex and incompletely understood. For purposes here, some parameters that comprise inputs to 'signal strength' are the density and affinity of antigen that is engaged by the TCR, the frequency of this engagement, and the costimulation and the amount and types of inflammatory cytokines present during antigenic encounter [5,6,51].

Caveats and clinical implications

The developmental model should not be over interpreted or treated as some orthodox dogma. We explicitly do not want to minimize the potential importance of dedifferentiation of T cells in the physiologic and non-physiologic settings. This is important when considering possible T cell de-differentiation when T cells experience situations in vivo where certain niches are unoccupied [55], especially as a result of certain disease conditions, chemotherapy or total body irradiation. Moreover, T cell differentiation should not be confounded with the 'off-on-off' changes that are associated with T cell activation, clonal expansion and quiescence of memory T cell subsets. Clearly, cells must experience transient changes in the metabolism of a T cell that enables clonal expansion such as the generation of 'building blocks' including the synthesis of nucleic acid precursors, organelles, membranes and other cellular components involved in T cell mitosis [56,57].

A deeper understanding of T cell differentiation is paramount for the development of effective vaccines and therapies that rely on memory $CD8^+$ T cell formation. In the setting of metastatic malignancy, clinical trials have already shown that factors associated with objective response include longer telomeres of the infused cells [58,59], the number of $CD27^+$ CD8 T cells infused [59,60], and the persistence of the cells in the circulation one month after transfer [59,61]. These observations are all consistent with the notion that transferred cells with a less differentiated phenotype are associated with a greater likelihood of objective response in adoptive cell transfer, findings that are corroborated in pre-clinical work [26,27,42,62-65]. Experiments using new techniques and even carefully-designed clinical trials may begin to resolve the long-standing debate about the lineage relationship between effector and memory T cells.

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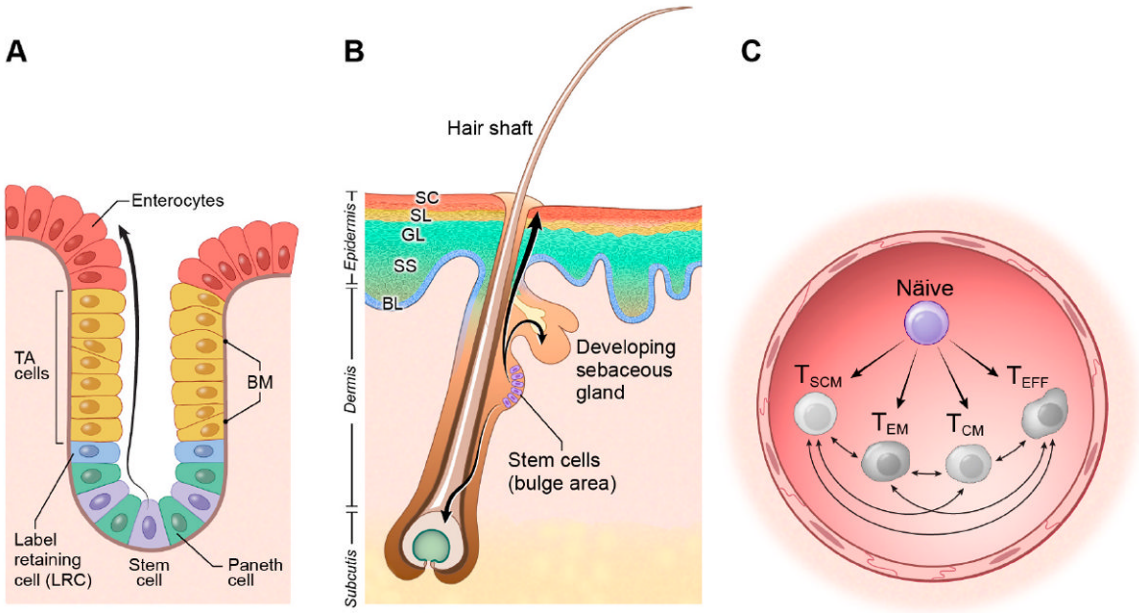

Figure 1. The linage relationship of T cell subsets is complicated by the lack of anatomical clues

A) The intestinal crypt-villus unit. Intestinal stem cells reside at the base of the crypt between Paneth cells. As cells proliferate and differentiate into transient amplifying (TA) progenitor cells and mature enterocytes, they move upwards to cover the villus. **B**) The skin. Epidermal stem cells are located in the bulge region of the hair follicle, the base of the sebaceous gland, and the basal layer of the interfollicular epidermis. As cells proliferate and differentiate into keratinocytes, they move upward to form the stratum spinosus (SS), the granular layer (GL), the stratum lucidum (SL) and the stratum corneum (SC). **C**) T cells. Following antigen-stimulation, naïve T cells differentiate generating the full diversity T cell subsets. The existence of cells at different developmental stages, all of which are moving within the same anatomical space, does not offer an easy static snapshot that provides clues about their lineage relationships which are still the subject of controversy in the field.

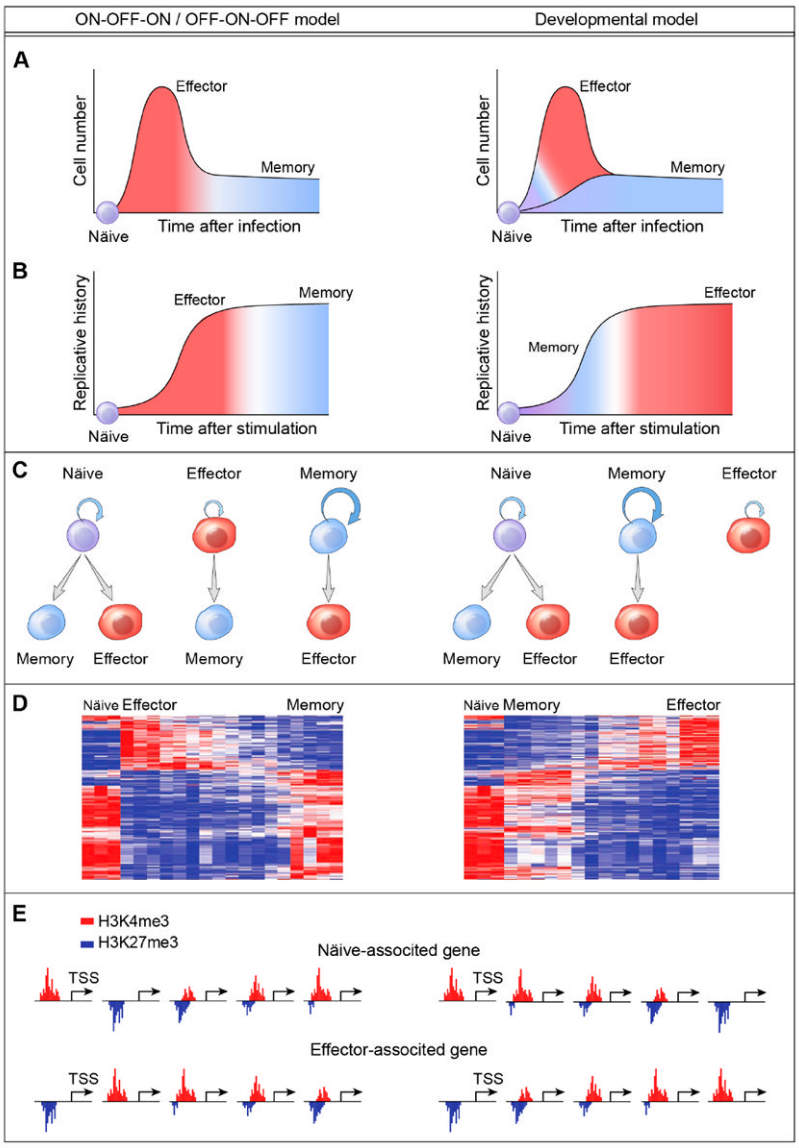

Figure 2. There are two dominant competing models of T cell differentiation

Set of panels shown on the left side depicts the 'off-on-off' model, in which all memory T cells are derived from cells that were once effector cells. Shown in the right set of panels is the 'developmental' model whereby memory cells arise directly out of naïve precursors without going through an effector stage. These distinct models lead to different predictions, which are explained in detail in the text.

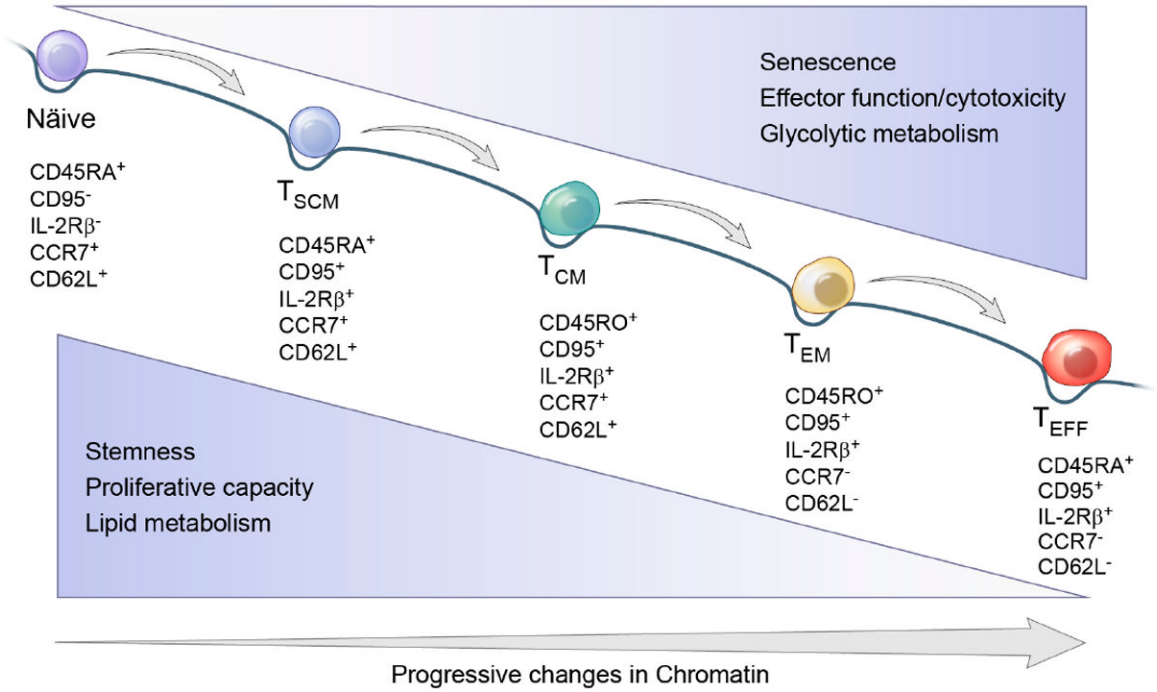

Figure 3. The progressive differentiation CD8$^+$ T cell differentiation is largely a linear and unidirectional process

The differentiation of cells proceeding efficiently in mainly one direction has been analogized to a ball rolling down a hill, with the gradual loss of potential. For T cells, this process leads to characteristic changes in cell surface molecules shown. In addition, the model includes observations that cellular differentiation, ie the acquisition of effector functions, is eventually accompanied by senescence. At the same time, there is a loss of 'stemness' – the capacity of cells to be multipotent and self-renewing – as well as a diminution in proliferative capacity. Cells move from a metabolism that is based on lipid oxidation and oxidative phosphorylation to one based on glycolysis.