The memory of a killer T cell: models of CD8+ T cell differentiation

Bram Gerritsen1 and Aridaman Pandit1,2

CD8+ T cells have an important role in protection against infections and reinfections of intra-cellular pathogens like viruses. Naive CD8+ T cells circulating in blood or lymphoid tissues can get activated upon stimulation by cognate antigen. The activated T cells undergo rapid proliferation and can expand more than 104-folds comprising largely of effector T cells. Upon antigen clearance, the CD8+ T-cell population contracts due to apoptosis, leaving behind a small population of memory T cells. The timing and mechanisms underlying the differentiation of naive cells into effector cells and memory cells is not yet clear. In this article, we review the recent quantitative studies that support different hypotheses of CD8+ T-cell differentiation.

INTRODUCTION

The adaptive immune system has two key roles: (a) to mount a rapid response in case of reinfections, and (b) to form a long-lived immunological ‘memory’ to mount a rapid response in case of re-exposure to the cognate antigen. Understanding immunological memory is important as it forms the basis of vaccination. Thucydides, in fifth century BC, first acknowledged the concept of immunological memory noting that the individuals who had recovered from plague were best able to help the sick as they themselves were not fatally reinfected. The earliest documented examples of procedures similar to modern day vaccination are from 17th century India and China. However, Edward Jenner demonstrated in 1796 that individuals inoculated with materials obtained from cowpox lesions were protected against the deadly smallpox virus. The success of smallpox vaccine subsequently paved the way for development of vaccines against other pathogens. However, there are many pathogens against which we do not currently have an effective vaccine. Our inability to develop vaccines against a wide range of pathogens using the classical methods indicates a need to better understand the mechanisms by which the adaptive immune system forms memory.

CD8+ T cells, also known as cytotoxic T lymphocytes or killer T cells, form an integral part of the adaptive immune system especially to respond to intracellular pathogens like viruses. In a seminal work, Zinkernagel and Doherty using lymphocytic choriomeningitis virus infection in mice established that T cells recognize viral antigens in association with self-major histocompatibility complex molecules, introducing the concept of ‘altered self’. Subsequently, it was established that each T cell expresses a unique T-cell receptor (TCR) that can bind to cognate peptide-bound major histocompatibility complex molecules expressed on the surface of antigen-presenting cells. TCRs are enormously diverse (1015 distinct TCRs are possible) and allow CD8+ T cells to mount a specific response against foreign antigens. CD8+ T cells can undergo massive expansion in response to foreign antigens. For example, Epstein-Barr virus infection typically results in swollen lymph nodes and an increase of peripheral blood monocyte count due to rampant proliferation of Epstein-Barr virus-specific CD8+ T cells. Advancements in the tetramer straining, adoptive transfer of CD8+ T-cell clones, and TCR transgenic mice helped in understanding antigen-specific expansion of CD8+ T cells during different stages of infection.

During primary infection, naive CD8+ T cells (T_N) are activated when they recognize their cognate antigen presented by dendritic cells in the spleen or in the draining lymph nodes. Upon activation, antigen-specific CD8+ T cells undergo clonal expansion and become effector CD8+ T cells (T_E). Incredibly, activated CD8+ T cells can undergo as many as 19 divisions in a week giving rise to 104 to 106 clones. Clonal expansion of CD8+ T cells is typically accompanied by reduction and ultimately eradication of the virus. The memory T cells are self-renewing multipotent cells that can persist in an antigen-independent manner for a long time. Upon reinfection and hence re-exposure to the cognate antigen, memory T cells exhibit high proliferative capacity. However, the timing and differentiation of effector and memory T cells has been among the most actively debated topics in immunology. Here, we will review the pathways and models of CD8+ T-cell differentiation into effector and memory phenotypes.

NAIVE CD8+ T CELLS

An individual can contain more than 1012 CD8+ T cells out of which about 100–1000 naive cells can mount a response against an antigen. To solve the complex puzzle of finding the T cells specific for the...
antigen, immune system facilitates constant recirculation and homing of T cells. In fact, blood-borne circulating naïve T cells enter the secondary lymphoid organs to scan for the cognate antigen and return to the circulation in approximately 10–20 h.13,14 Several molecules expressed on the surface of naïve T cells help them to migrate to lymphoid tissue. Once activated, the CD8+ T cells proliferate and migrate to the site of infection. CD8+ (and CD4+) T-cell subsets express different surface molecules that help them to perform different functions and to home to different tissues.15 Some surface molecules have been used as markers to distinguish between different subsets because surface molecules correlate with functional properties of CD8+ T-cell subsets.

To discover cognate antigen, naïve T cells recirculate through the secondary lymphoid organs and express molecules that help in lymph node homing. To enter the lymph nodes and overcome the shear forces in blood, naïve CD8+ T cells first tether to the high endothelial venules using CD62L (L-selectin). CC-chemokine receptor 7 (CCR7) is a G-protein coupled chemokine receptor that recognizes CCL21 (or CCL19) ligand immobilized on the high endothelial venules. CCR7-mediated signaling activates LFA1 (lymphocyte function-associated antigen 1), which binds ICAM1 (intercellular adhesion molecule 1), arresting the naïve T cell and facilitating migration into the lymph node. Thus, CD62L and CCR7 are important lymph node homing markers. Interestingly, inflammatory signals in the lymph node induce CCR5 expression on the naïve CD8+ T cells to facilitate migration toward antigen-presenting dendritic cells and interacting CD4+ T cells.15–17

DIRECTIONS FOR THE KILLER T CELLS

Upon activation, TCR-mediated signaling induces transient upregulation of CCR7 to retain the activated T cell in the lymph node. The retention in the lymph node provides the activated CD8+ T cell essential cytokines and co-stimulatory signals to facilitate rapid proliferation. The primary goal of the activated CD8+ T cells is to eradicate infection. For CD8+ T cells, strength lies in numbers as more than 3 × 10^6 antigen-specific CD8+ T cells might be required for effective viral and tumor regression.18 Massive clonal expansion in lymph nodes generates a large number of CD8+ T cells with effector phenotype. These effector cells perform antigen-specific lysis of infected cells by exocytosis of granules containing perforin, granzyme A and granzyme B.18–20 Interleukin-12 produced by phagocytes and dendritic cells promotes cytotoxicity of effector T cells and promotes production of interferon-γ and tumor necrosis factor.19,20 Because \( T_E \) cells can perform their effector functions only locally, they migrate to the site of infection. To egress out of the lymph nodes, CD62L and CCR7 are downregulated in effector CD8+ T cells.16,21 Migration of the effector cells to the sites of infection is a complex procedure. It has been hypothesized that tissue specific ‘area codes’ or combination of selectins, cytokines and integrins act as homing markers and help the effector cells to reach the infected tissue.16 T cells activated in the skin-draining lymph nodes exhibit preferential upregulation of one or more skin homing surface molecules like CCR4, CCR10, E-selectin and P-selectin.15,22 Similarly, T cells activated in the gastrointestinal-associated lymphoid tissue exhibit preferential upregulation of small intestinal mucosa homing surface molecules like α4β7 and CCR9.15,23 Several lines of evidence ascertain that non-lymphoid tissue homing is multi-factorial, complex, and is largely dictated via ‘area codes’; however, a small fraction of effector T cells can end up in uninfected tissues. Survival, division and differentiation of \( T_E \) cells may further depend on secondary encounters with the cognate antigen in the infected tissue.24 Thus, effector CD8+ T cells display different markers depending on their activation and site of infection.

DIFFERENT LOCATION DIFFERENT MEMORIES

CD45 gene products, splice variants CD45RA and CD45RO, were classically used as markers to distinguish between naïve and memory T cells. CD45RO (lower molecular weight) is preferentially expressed on the memory T cells, whereas CD45RA (higher molecular weight) is preferentially expressed on the naïve T cells. In a seminal study, Sallusto et al.25 established that the memory T cells exhibit heterogeneity in their homing markers and classified two distinct memory subsets for CD8+ (and CD4+) T cells: central memory (TCM) and effector memory (TEM) T cells. TEM cells, like \( T_N \) cells, are CD62L+ and CCR7+ allowing them to recirculate through the lymph nodes. TCM cells are self-renewing, have the capacity to produce high amounts of interleukin-2, and have the potential to differentiate into effector phenotypes upon re-stimulation with the cognate antigen.15,25–27 So, TCM cells are the long-lived memory T cells, residing in the secondary lymphoid organs with a potential to mount a rapid response upon reinfection.

TCM cells, unlike TCM cells lack lymph node homing markers, that is, they are CD62L− and CCR7−. TEM cells, typically found in the non-lymphoid tissues, exhibit effector-like functions and have the capacity to produce interferon-γ and tumor necrosis factor.25,26,28,29 TEM cells undergo limited homeostatic turnover and express low to intermediate levels of co-stimulation and pro-survival markers like CD127 (interleukin-7 receptor), CD122, PD-1, CD28 and so on.26 Similar to effector CD8+ T cells, TEM cells exhibit different combination of selectins, cytokines and integrins that help them to home to different non-lymphoid tissues. Different localization and cytolytic activity of TCM and TEM cells indicate division of labor in memory T cell subsets. TEM cells, dwelling in the peripheral tissues, mount a rapid initial response to check the replication and spread of the invading pathogen. Meanwhile, TCM cells can undergo rapid proliferation and differentiation into effector cells to mount a robust response. Additional memory subsets (TEMRA, TEM and so on) can be defined based on the tissue localization and homing markers (see Mahnke et al.26 for a detailed review).

MODELS OF T-CELL DIFFERENTIATION

Mathematical modeling has played an essential role in understanding and quantifying complex biological systems like the immune system.30,31 Mathematical models typically try to understand the
complex biological interactions by using simple, yet informative, toy models. An important goal of mathematical models is to arrive at the simplest plausible explanation for biological data. Mathematical models can be used to make experimentally testable predictions based on different hypotheses. Additional experiments can then help in rejecting a hypothesis leading to improvements in the model. With the advent of high-throughput techniques and carefully designed experimental systems, immunology is becoming more and more quantitative. Mathematical models have been vital to explain and establish several fundamental immunological questions like rates of clonal expansion, immunodominance, lymphocyte division and death rates and so on.\(^1\)\(^{30}\) The timing and differentiation of memory T cells had been a topic of several discussions. Mathematical models have also been used to test various hypotheses for CD8\(^+\) T-cell differentiation.\(^32\)\(^{–}\)\(^34\) The three most prominent hypotheses assume linear differentiation for CD8\(^+\) T cells:

- **Effector first hypothesis**: Naive CD8\(^+\) T cells first differentiate into effector and later into memory CD8\(^+\) T cells (Figure 2a).
- **Decreasing potential hypothesis**: Naive CD8\(^+\) T cells differentiate into activated cells with memory potential and later differentiate into effector CD8\(^+\) T cells (Figure 2b).
- **Asymmetric division hypothesis**: Naive CD8\(^+\) T cells upon first division produce one daughter cell with propensity to produce memory cells and another with propensity to produce effector cells (Figure 2c).

The three hypotheses thus indicate a contrast in the timing of memory formation. These hypotheses can be framed in terms of simple ordinary differential equations as follows:

\[ \frac{dT_E}{dt} = \rho T_E - (r + \delta_E) T_E, \]

\[ \frac{dT_M}{dt} = r T_E - \delta_M T_M. \]

\[ \frac{dT_E}{dt} = \rho T_E - (r + \delta_E) T_E. \]

\[ \frac{dT_M}{dt} = r T_E - \delta_M T_M. \]

Similarly to Boer et al.,\(^32\) we can assume that at the start of the expansion phase naïve cells have differentiated to give a small number of effector cells. During the expansion phase, effector cells then proliferate at rate \( \rho \). Effector cells differentiate into memory cells at rate \( r \) or die at rate \( \delta_E \). Memory cells die at rate \( \delta_M \). Alternatively, we can frame a simple model for decreasing potential hypothesis as described in Kohler: \(^33\)

\[ \frac{dT_E}{dt} = \rho T_E - (r + \delta_E) T_E. \]

\[ \frac{dT_M}{dt} = r T_E - \delta_M T_M. \]

During the expansion phase, memory cells proliferate at rate \( \rho \), differentiate into effector cells at rate \( r \), and die at rate \( \delta_M \). Similarly, effector cells proliferate at rate \( \rho \) and die at rate \( \delta_E \). Here, Kohler considered that at the start of the expansion phase only memory (\( T_M \)) cells are present which then later differentiate into effector T cells (\( T_E \)). Similarly, we can frame the asymmetric division hypothesis:

\[ \frac{dT_E}{dt} = \rho T_E - \delta_E T_E, \]

\[ \frac{dT_M}{dt} = \rho M - \delta_M T_M. \]

For asymmetric division hypothesis, the effector cells proliferate at rate \( \rho_E \) and die at rate \( \delta_E \), while the memory cells proliferate at rate \( \rho_M \) and die at rate \( \delta_M \). Interestingly, all effector first, decreasing potential, and asymmetric division hypothesis could explain the response mounted against lymphocytic choriomeningitis virus infection with biologically realistic parameters (Figure 3).\(^32\)\(^,\)\(^33\) The three models differed in their predictions of memory T-cell numbers during the course of the infection which is an experimentally testable prediction. The effector first hypothesis proposes that the fraction of memory cells either increases or remains constant during the expansion phase (Figure 3b). Whereas the decreasing potential and asymmetric division hypotheses propose that the fraction of memory cells decreases during the expansion phase (Figure 3b). Experimental studies have shown that the majority of CD8\(^+\) T cells at the peak of the response exhibit effector phenotype (CD62L\(^-\) CD44\(^+\)). Although accurate quantification of CD62L\(^+\) vs CD62L\(^-\) T cells is difficult, single-cell studies show that about 10% T cells remain CD62L\(^+\) during late infection.\(^35\)\(^,\)\(^36\) To differentiate between these models, accurate temporal and phylogenetic profiling of clonally expanding T cells is required.

**FATE OF INDIVIDUAL NAIVE CELLS**

To achieve a robust response against an antigen, individual T\(_N\) cells may follow a defined linear differentiation pathway producing effector and memory cells. Alternatively, individual T\(_N\) cells can follow different differentiation pathways where some T\(_N\) cell preferentially differentiate into memory T cells, while others preferentially

---

**Figure 2** Different hypotheses for timing of CD8\(^+\) T cell memory formation. (a) For effector first hypothesis, naïve CD8\(^+\) T cells (gray) differentiates into clonally expanding effector CD8\(^+\) T cells (CD62L\(^-\)CCR7\(^-\)CD27\(^-\) red), some of which survive after the clearance of infection as long-lived memory CD8\(^+\) T cells (CD62L\(^-\)CCR7\(^-\)CD27\(^-\) blue). (b) For decreasing potential hypothesis, naïve CD8\(^+\) T cells (gray) first differentiate into memory precursor CD8\(^+\) T cells (blue), which further differentiate into effector CD8\(^+\) T cells (red). (c) For asymmetric cell division hypothesis, naïve CD8\(^+\) T cells (gray) divide and differentiate into two daughter T cells of which one has a propensity to become a memory CD8\(^+\) T cell and the other has a propensity to become an effector CD8\(^+\) T cell. Dashed lines indicate that a fraction of cells from one cell type divide and differentiate into another cell type. A full color version of this figure is available online at the [Immunology and Cell Biology website](https://www.immunologycellbiology.com).
differentiate into effector phenotypes. Elegant single-cell tracing experiments using adaptive transfer of barcoded cells or congenic marker bearing cells in mice have confirmed that individual naive CD8+ T cell can produce both effector and memory phenotypes.\textsuperscript{10,34,37} Using DNA barcode based in vivo lineage tracing, Gerlach et al.\textsuperscript{10} found that progenies of individual T\textsubscript{N} cells contribute differentially toward effector and memory phenotypes. Gerlach et al.\textsuperscript{10} used barcoded cells with OT-I TCR that recognize SIINFEKL peptide presented on H-2K\textsuperscript{b} and found that individual naive T cells exhibited different family sizes (that is, different number of progenies). Interestingly, a few individual naive cells produced as many as $10^5$ progenies, while majority of individual naive cells produced $> 200$ progenies. There exists a division of labor where most naive T cells produce smaller families in which a larger fraction of cells have memory potential (CD62L\textsuperscript{+} CD27\textsuperscript{+} KLRG-1\textsuperscript{-}), whereas few naive T cells produce larger families in which a larger fraction of cells have effector potential (CD62L\textsuperscript{-} CD27\textsuperscript{-} KLRG-1\textsuperscript{+}).

Adoptive transfer of cells with OT-I TCR expressing different congenic markers (CD45 and/or CD90) and mathematical modeling showed that stochastic factors determine the division rate of individual T cells.\textsuperscript{34} Buchholz et al.\textsuperscript{34} performed depth fate mapping of individual naive T-cell progenies using CD62L and CD27 markers. They divided progenies from individual naive T cells into three phenotypes using the expression profile of two markers: central memory precursors (CD62L\textsuperscript{+} CD27\textsuperscript{+}), effector memory precursors (CD62L\textsuperscript{-} CD27\textsuperscript{+}) and effector cells (CD62L\textsuperscript{-} CD27\textsuperscript{-}). Buchholz et al.\textsuperscript{34} performed comprehensive mathematical analysis to test 304 models of $T_{CM}$, $T_{EM}$ and $T_{E}$ differentiation and found that linear differentiation model of decreasing potential (that is, naive to memory precursors to effectors) best explained their in vivo data. Thus, single-cell lineage and fate tracing studies indicate that cells undergoing fewer divisions, and hence exhibiting smaller family sizes, produce more cells with memory phenotype.

**ASYMMETRIC DIVISION**

CD8+ T cells can express a wide array of lymphoid and non-lymphoid homing markers depending upon their activation and stimulation environment. Thus, quantification of multiple markers is required to accurately distinguish between $T_{CM}$, $T_{EM}$ and $T_{E}$ phenotypes.\textsuperscript{15,26,58}

Arsenio et al.\textsuperscript{39} used cell sorting and gene expression assays to study individual CD8+ T cells over the course of infection. From 94 gene expression profiles of 1300 single-cells extracted at different days and divisions post infection, they found that $T_{N}$, $T_{CM}$, $T_{EM}$ and $T_{E}$ cells form distinct clusters, thus exhibiting different gene expression profiles. Interestingly, their multivariate analysis showed that CD8+ T cells at day 1, 3 and 5 exhibit gene expression profiles distinct from $T_{N}$, $T_{CM}$, $T_{EM}$ and $T_{E}$ cells. Multiple studies indicate that activated CD8+ T cells exhibit heterogeneous gene expression profiles, and may not have committed to a single fate.\textsuperscript{39,40} Intermediate heterogeneity indicates that commitment of fate of the individual T cell might not occur in the starting. However, Arsenio et al.\textsuperscript{39} using a statistical Markov model suggested that upon activation T cells may acquire a propensity toward either effector or memory phenotype.\textsuperscript{39} They propose that a $T_{N}$ cell undergoes asymmetric division where proximal daughter cell exhibits higher propensity toward effector phenotype, whereas a distal daughter cell from the same mother cell exhibits higher propensity toward memory phenotype. Interestingly, reanalysis of the data using unsupervised clustering algorithms showed that further experiments are needed to resolve between asymmetric division and decreasing potential models.\textsuperscript{41}

**WHERE DO WE STAND?**

Activated T cells exhibit large heterogeneity, a virtue of cell intrinsic and/or cell extrinsic factors, leading to disparate response against the antigen. Cell intrinsic heterogeneity can be introduced before T cells are activated (that is, asymmetry in the population of antigen specific T cells).\textsuperscript{42} Alternatively, differences in progenies of an individual CD8+ T cells can arise at or after activation (that is, asymmetry in the fate of daughter T cells). Advent of elegant techniques like adoptive transfer, cell sorting, cellular barcoding, mouse models and so on, suggest that individual naive CD8+ T cells can produce a heterogeneous population comprising effector and memory phenotypes.\textsuperscript{10,34,37,39,40,43} At least three major hypotheses have been proposed for naive CD8+ T-cell differentiation differing majorly in the timing of commitment to memory fate. Mathematical models constructed using different hypotheses were consistent with the experimentally observed antigen response. The inability to discriminate between different models of
CD8+ T-cell differentiation has been one of the most debated topics in immunology.

The effector first hypothesis has teleological appeal and several studies have demonstrated that memory T cells can be derived from cells that transcribed effector specific genes. In early experiments, adoptive transfer of proliferating effector CD8+ T cells into mice displayed the potential to form memory cells and supported the effector first hypothesis.37,44 However, one cannot negate the fact that the proliferating effector CD8+ T-cell population before adoptive transfer could contain cells with propensity to acquire memory phenotype. Subsequently, it was shown that adoptive transfer of perforin or granzyme B producing CD8+ T cells also displayed the potential to form memory cells.57,45,46 Furthermore, to exclude the possibility of contamination in adoptively transferred cells, reporter mouse strains were used to show that memory CD8+ T cells can arise from T cells that once transcribed interferon-γ or granzyme B genes.37,48

The decreasing potential hypothesis is similar to a secondary response wherein self-renewing memory T cells proliferate and differentiate into effector T cells. Strong evidence for the decreasing potential hypothesis comes from the fact that memory T cells have longer telomeres and more active telomerase compared with the effector T cells.2,25,49,50 Thus, it is less likely that effector T cells (with shorter telomeres) differentiate into memory T cells (with longer telomeres). In addition, during primary infection, repeated antigenic stimulation differentiates into memory T cells.52 Moreover, blocking effector differentiation pathways generally results in differentiated cells with memory phenotype.2,53

A recent observation that memory precursor cells are as potent as effector cells in terms of effectors functions (cytotoxic potential, degranulation and so on) may reconcile in part the differences between effector first and decreasing potential hypotheses.54 Upon CD8+ T-cell differentiation, cytokine environment and secondary antigen exposure in inflamed sites boosts the effector potential, while cells localized in secondary lymphoid tissues exhibit less effector functions despite having the potential to do so. It is now becoming increasingly apparent that number of divisions may strongly influence the expression of secondary lymphoid tissue homing markers like CCL21,34,35,55,56 The slow dividing cells (that is, CD62L+ CCR7+ cells) that preferentially home to the lymphoid tissues continue to receive survival signals from the lymphoid environment and thus can survive longer than fast dividing non-homing cells. Nevertheless, the potential of CD62L+ high cells to display effector functions and the capability of effector cells to produce memory cells indicate that the localization of the activated T cell may dictate the fate. Furthermore, it is not clear if the fate of the CD8+ T cells is fixed or can display plasticity. Further studies that comprehensively map the role of stimulatory environment, number of divisions on homing marker distributions, antigen stimulation and so on, are required to delineate the discrepancies in the timing of CD8+ T-cell fate commitment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

AP was financially supported by the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement 317040 (QuanTI). BG was financially supported by the VIRGO consortium, which is funded by The Netherlands Genomics Initiative and by the Dutch government (FES0908).


48 Bannard O, Kraman M, Fearon DT. Secondary replicative function of CD8+ T cells that had developed an effector phenotype. Science 2009; 323: 505-509.


52 D’Souza WN, Hedrick SM. Latecomer CD8 T cells are imprinted with a unique differentiation program. J Immunol 2006; 177: 777-781.


