A Closer Look at Opposing Models for the T Cell Response to Pathogens

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Abstract. The problem of understanding the mechanisms of differentiation, activation, and interconversion of phenotypes of $CD8^+$ T cells is one of crucial importance in cancer therapy, owing to both the anti-tumor efficacy of $CD8^+$ T cells as well as the severe toxicity that results from excess expansion of this population. Several opposing theories exist which describe potential pathways for the development of the $CD8^+$ T cell repertoire; however, the accuracy of each remains controversial. Here we review the current hypotheses, provide a critical overview of pivotal biological data from which these theories are derived, and discuss principle population-level implications. Finally, we offer a novel hypothesis which maintains consistency with each of the experimental studies and seeks to unify the currently opposing but not so disparate theories.

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Introduction and Background

The importance of understanding T cell differentiation has been recognized, not only in the basic sciences, but also in translation and medical sciences, particularly by those interested in the development and optimization of immunologically based therapies for the treatment of cancers and autoimmune diseases. The success of these therapies relies on precise optimization of the anti-tumor immune response, at the crux of which is $CD8^+$ T cell activation and differentiation. Specifically, upon activation by antigen presenting cells, naïve (antigen-inexperienced) $CD8^+$ T (T_N) cells give rise to progeny which have both effector and memory phenotypes. Effector (T_E) cells reside primarily in peripheral tissues and are characterized by having high cytotoxicity and decreased longevity. Memory (T_M) cells exhibit stem-cell-like longevity and are further divided into two subtypes, central memory (T_{CM}) cells, which reside primarily in lymphoid tissue and have limited cytoxicity, and effector memory (T_{EM}) cells, which reside primarily in peripheral tissues and have intermediate cytotoxicity [1, 2, 3, 4, 5]. Due in part to variation in classification procedures between laboratories, the paths by which these populations interconvert have become the subject of much "chicken or the egg" type debate [1, 2, 6, 7, 8, 9].

Presently, the debate centers predominantly around four models for $CD8^+$ T cell activation and differentiation: (1) the Linear or Uniform Potential Model, in which T cells differentiate in the order $T_N \to T_E \to T_M$, (2) the Progressive or Decreasing Potential Model, in which T cells differentiate $T_N \to T_{CM} \to T_{EM} \to T_E$, (3) the Signal Strength or Fixed Lineage Model, in which T_N cells differentiate either to T_E cells, in the case of high antigen signal, or to T_M cells in the case of low antigen signal, and are thereafter restricted respectively to the effector or memory phenotype, and (4) the Asymmetric Division Model, in which T_N cells give rise to two distinct daughter cells, one with a greater effector potential and one with a greater memory potential [2].

Several major hurdles preclude a straightforward understanding of this process, not least of which is ambiguity in classification of T cell subtypes. Standard procedures for identifying T cell populations rely on flow cytometry to measure concentrations of surface markers on individual cells. From this output, cells are sorted by "high" or "low" expression levels of particular surface markers, and classified based on the combinations of these markers they express. Discrepancy in classification thus arises due to (a) subjectively defined boundaries used to delimit "high" or "low" expression levels, which can lead to large discrepancies in measurement when subpopulations are not clearly delineated, as is known to be the case especially in the early immune response [10], and (b) variation in the combinations of markers used to classify cells, which can result in non-trivial overlap of populations identified as discrepant phenotypes. For example, while T_N cells are frequently sorted as $CD62L^+CD44L^-$, under certain conditions, T_M differentiation can be halted, resulting in the formation of a $CD62L^+CD44L^-$ T stem cell memory (T_{SCM}) population, which can be distinguished from the T_N population by their expression of CD95 (as well as CXCR3, IL-2Rβ, or LFA-1) [4]. However, cells are not always gated on these particular surface markers, and hence,

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$CD62L^+CD44L^-CD95^+$ memory T cells are often inadvertently classified as T_N cells.

Table 1 provides a summary of surface marker profiles characteristic of T_N cells as well as four additional phenotypes to which T_N cells are known to differentiate through one mechanism or another: T_{CM}, T_{EM}, early effector (T_{EE}), and short-lived effector (T_{SLE}) cells. We remark that while the expression profiles in Table 1 do not represent an exhaustive list of possible phenotypes (as this would require an encoding with a dimension equivalent to the number of known surface markers), the expression profiles listed are sufficient to fully distinguish the populations considered. We further remark that various intermediates are indeed known to exist, which will not be considered here, including, at least, T_{SCM} cells, mentioned above, and also intermediate memory T_{IM} [11].

TABLE 1. Typical surface marker characterizations of $CD8^+$ T cells. *Abbreviations*: T_N, naïve; T_{CM}, central memory; T_{EM}, effector memory; T_{EE} early effector cell; T_{SLE}, short-lived effector.

	CD44	CD62L L-Selectin	CCR7	GrzB	KLRG-1	IL-7Rβ CD127	CD11a	CD45RO	CD45RA
T_N	-	+	+	-	-	+	-	-	+
T_{CM}	+	+	+	-	-	+	+	+	-
T_{EM}	+	-	-	-	lo	+	+	+	-
T_{EE}	+	-	-	+	lo	-	+	-	+
T_{SLE}	+	-	-	+	hi	-	+	-	+

Restricting our focus, however, to the subtypes characterized in Table 1, there exist 24,010 possible combinations of subtype conversion pathways which satisfy the following conditions: (1) the T_N subtype has no inward-directed paths (based on the widely agreed upon view that activated T cells do not revert to a naïve phenotype after sufficient encounters with antigen), and (2) the remaining four subtypes each have zero (corresponding to terminal differentiation), one (corresponding to symmetric division), or two (corresponding asymmetric division), outgoing paths. A schematic representation of the possible pathways is shown in Figure 1 (left). In this review we consider the possibility of existence of a subset of these pathways which remain consistent with all each of the experimental studies reviewed.

The aim of the remainder of our discussion is to establish the possibility of consistency of the predominant biological data presented in support of opposing views by considering the existence of a subset of these pathways which cannot be ruled inconsistent with the literature. We contend that our inclusion of only a subset of possible T cell phenotypes is justifiable given that (a) the existence of non-terminal phenotypes does not preclude identification of interconversion pathways between phenotypes, and (b) the existence of unidentified terminal phenotypes corresponds to irreversible loss of T cells from the total subset of populations considered, which is already an inherent possibility due to the potential for loss of a paricular lineage upon cell death.

Review of existing biological data.

Change et al., 2007

We first consider the 2007 Chang et al. study [3] in which T cells were sorted via flow cytometry and directly visualized using confocal microscopy. In this study, labeled, naïve T cells were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) and adoptively transfered into wild-type mice infected with Listeria monocytogenes. Subsequently, cells were sorted by relative concentrations of CFSE to identify activated T cells undergoing or having undergone their first division. Polarization of surface markers was observed in greater than 90% of premitotic cells, this polarization was identified to be dependent on the location of the immune synapse, and the proximal and distal daughter cells which were produced after first division were identified to be $CD62L^-CD44^+GrzB^+IL\text{-}7R\alpha^-$ and $CD62L^+CD44^-GrzB^-IL\text{-}7R\alpha^+$. Thus, the phenotype of the distal daughter is consistent with that of a T_{CM} cell, and the proximal daughter cell phenotype is consistent with either T_{EE} or T_{SLE} cells. Further, because a cell which expresses no KLGR-1 must first express intermediate levels of KLRG-1 before expressing high levels, by necessity of the intermediate value, it is natural to conclude that the proximal daughter arising from the first division after activation of a naïve T cell is most appropriately classified as a T_{EE} cell (this of course is consistent with the naming convention adopted throughout the field). The possible differentiation pathways which satisfy these conditions are now reduced to those shown in Figure 1 (left center), where solid lines denote pathways known to exist, and red lines denote pathways known to rely on activation by antigen presenting cells.

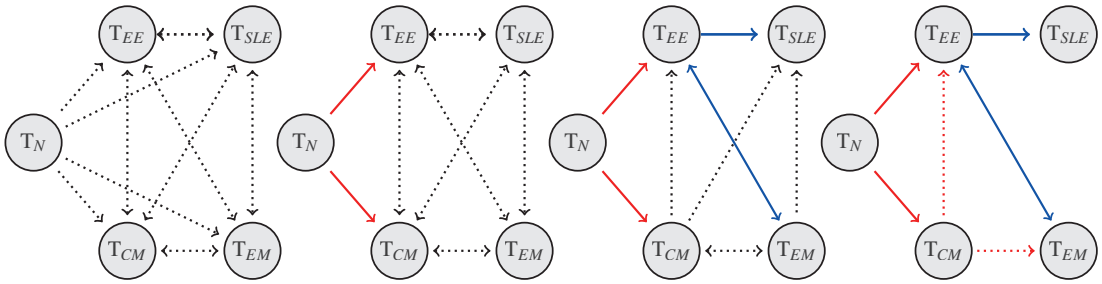

FIGURE 1. Schematic representations of *(left)* all possible paths for interconversion of T cell phenotypes assuming initial activation of T_N cells is irreversible, *(left center)* pathways consistent with results from [3], *(right center)* pathways with [3] and [10], and *(right)* pathways consistent with [3, 10, 11, 12]. Solid lines represent confirmed existence of pathways, dotted lines represent pathways not contradicted. Red lines represent pathways along which cells progress upon activation by antigen presenting cells. Blue lines represent pathways along which cells are proposed to progress due to antigen concentration and inflammatory signals which may be independent of immune synapse formation.

Sarkar et al., 2008

We next consider a 2008 study by Sarkar et al. [10] in which both KLRG-1^- and KLRG-1^+ cells were transferred to donor mice. In the former case, almost 20% of the donor cells survived 40 days after transfer, whereas in the latter case less than 2% of donor cells persisted. We note that in this study in particular, a large percentage of cells exists near the boundary distinguishing KLRG-1^+ and KLRG-1^-. This indicates that cells expressing high amounts of KLRG-1, namely T_{EE} cells, are likely a terminally differentiated population.

Plumlee et al., 2015

Further, a 2015 study by Plumlee et al. [12] in which a KLRG-1^-CD127^- population, identified as T_{EE} cells, were purified from virally infected donor mice and transferred to recipient mice which either were or were not previously infected. The recovered donor cells consisted of both KLRG-1^+CD127^- cells (T_{SLE}), and KLRG-1^-CD127^+ cells. However, the number of each KLRG-1^+CD127^- recoverd from virally infected recipients was significantly greater than the number recovered from uninfected recipients. Further, the KLRG-1 produced in recovered populations was greater than Combining the information from these two studies we conclude that T_{EE} cells can give rise to either T_M cells or T_{SLE} cells, and that T_{SLE} cells are likely terminally differentiated, bring us to the schematic represented in Figure 1 (right center). The blue line here is used denote differentiation pathways not known to necessary be linked to encounters with antigen presenting cells.

Unsoeld et al., 2005

We lastly consider a straightforward study published by Unsoeld et al. in 2005 in which labeled T cells from lymphocytic choriomeningitis virus (LCMV) infected mice were sorted via flow cytometry for CD62L$^+$CCR7^+ expression, a phenotype consistent with T_{CM} cells, purified, and transferred into B6 mice which were subsequently infected with LCMV. Following the infection, labeled donor T cells were sorted and identified to consist of both CD62L$^+$CCR7^+ and CD62L$^-$CCR7^- cells, which definitively establishes the existence of a path from T_{CM} cells to T_{EM} cells. Considering this information together with the fact that T_{CM} cells reside in the lymph nodes much like T_N cells, and are able to undergo subsequent activation by dendritic cells, we may guess that T_{CM} cells also undergo asymmetric division upon activation. We remark that, to our knowledge, while this has not been proven, it also has not been disproven. This may be a result of (a) difficulty in distinguishing cells which have undergone two division linked activations and cells which have undergoone one division linked activation and one homeostatic division, and (b) a smaller absolute difference in concentrations of surface markers between T_{EM} and T_{EE} cells than between T_{CM} and T_{EE} cells makes a definitive assessment of asymmetric division more difficult to obtain. Combining this information with that from previous studies, we arrive at the schematic presented in Figure 1 (left).

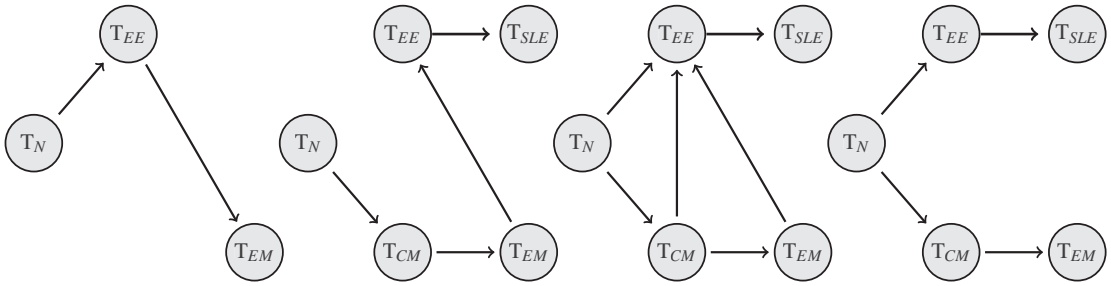

FIGURE 2. Subgraphs of schematic represented in Figure 1 (left), highlighting *(left)* the Linear Differentiation Model, *(left center)*, the Decreasing Potential Model, *(right center)* the Asymmetric Division Model, and *(right)* the Signal Strength Model.

Discussion

Interestingly, we see that the graphical representation of the differentiation pathways consistent with each of [3, 10, 11, 12], shown in Figure 1 *(left)*, contains subgraphs (Figure 2) which delineate the Linear Differentiation Model, *(left center)*, the Decreasing Potential Model, *(right center)* the Asymmetric Division Model, and *(right)* the Signal Strength Model. Thus, it seems at the very least possible that all of the currently opposing theories discussed here are in fact consistent with each other. On the other hand, (with careful attention paid to the direction of the edges) *none* of the schematics outlining the current theories capture *all* of the data described in this review independently. We conclude then that the existence of cell lineages which conform to any one of the present views is insufficient to rule out any of the others. It seems therefore pragmatic that future debate consider the possibility that these theories are in fact all in agreement, simply describing different, yet equally important, aspects of the T cell response to pathogens.

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