Review

CellPress

Single cell behavior in T cell differentiation

Jan C. Rohr[1,2], Carmen Gerlach[3], Lianne Kok[1], and Ton N. Schumacher[1]

[1] Division of Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands
[2] Center for Chronic Immunodeficiency (CCI), University Medical Center Freiburg and University of Freiburg, Freiburg, Germany
[3] Department of Microbiology & Immunobiology, Harvard Medical School, Boston, MA, USA

Upon primary infection, naïve T cells that recognize their cognate antigen become activated, proliferate, and simultaneously differentiate into various subsets. A long-standing question in the field has been how this cellular diversification is achieved. Conceptually, diverse cellular output may either arise from every single cell or only from populations of naïve cells. Furthermore, such diversity may either be driven by cell-intrinsic heterogeneity or by external, niche-derived signals. In this review, we discuss how recently developed technologies have allowed the analysis of the mechanisms underlying T cell diversification at the single cell level. In addition, we outline the implications of this work on our understanding of the formation of immunological memory, and describe a number of unresolved key questions in this field.

Diversification of T lymphocytes upon infection – fates and states

Whenever we encounter an infection, those few naïve T lymphocytes that recognize a pathogen-derived antigen are activated, expand, and in parallel, differentiate into subsets conferring different functions. A basic allocation of these subsets consists of cells with and without the potential to form immunological memory, so-called memory precursor effector cells (MPECs) and short-lived effector cells (SLECs), respectively. SLECs are considered to be terminally differentiated effector cells, which mediate pathogen eradication during the primary infection and then rapidly undergo apoptosis. By contrast, MPECs can differentiate into memory T cells that survive long term and provide protection upon renewed infection with the same pathogen. MPECs and SLECs are generally considered to represent distinct cellular fates, with fate being defined as a heritable state directing a cell and all its progeny to a particular phenotype or function [1]. Several cell-surface proteins have been put forward as markers to distinguish MPECs and SLECs. In particular, separation of activated T lymphocytes during infection based on expression of KLRG1 and CD127 has been shown to yield populations enriched in either SLECs or MPECs in adoptive transfer studies [65,69–71]. It should be noted though, that T cell diversification occurs on a much larger scale than just the ability to survive after pathogen clearance. These additional layers of diversification include phenotypes other than those defined by killer cell lectin-like receptor subfamily G, member 1 (KLRG1) and CD127, but also functional capacities and spatial distribution [5]. For example, memory T cells can be divided into central memory (T_{cm}), effector memory (T_{em}), tissue resident memory (T_{rm}), and bone-marrow memory T cells, based on their differential localization and migration behavior. In recent work, Newell and colleagues have utilized mass cytometry to examine the phenotypic and functional diversity of $CD8^+$ memory T cells at an unprecedented level. Simultaneous analysis of nine functional parameters revealed the occurrence of >200 different combinations of functions in $CD8^+$ memory T cells [6]. For $CD4^+$ T cells, similar analyses have thus far not been performed. However, in view of the variety of functional activities that $CD4^+$ T cells can display, a similar, if not even greater heterogeneity may be expected. Although the large heterogeneity observed in the mass cytometry study work may at first glance suggest a random acquisition of functional properties, the number of combinations observed for $CD8^+$ T cells is still well below the 512 ($=2^9$) combinations that are theoretically possible. This suggests that some functions of T cells are co-regulated [7], or – to put this in the language of Waddington's epigenetic landscape – that there are local minima in the landscape of all possible functional cell states, in which cells are more likely to be found. It is important to realize that these data reveal the existence of a large number of different T cell states; not necessarily T cell fates (described in greater depth in [1]): only a few T cell states have been shown to be permanently fixed under physiological circumstances, and may thus truly be considered fates. For example, T_{rm} $CD8^+$ T cells generally do not leave the peripheral tissue site in which they reside [8]. However, for many other T cell states described, evidence that these should be considered fixed properties, rather than transient conditions that can readily be left, is thin at best [1].

Regardless of whether a given acquired state is transient or permanently fixed, it is of interest to understand how T cell diversification arises in the first place. In the

Corresponding author: Schumacher, T.N. (t.schumacher@nki.nl).
Keywords: T cell differentiation; single-cell tracking; asymmetric cell division; population asymmetry; cellular heterogeneity; cell-extrinsic signals.

1471-4906/$ – see front matter
 http://dx.doi.org/10.1016/j.it.2014.02.006

subsequent paragraphs we first depict fundamental mechanisms for cellular diversification in any proliferating cell compartment. We then describe the evidence for or against these different mechanisms in the generation of a diverse T cell pool. Finally, we outline the implications of these recent data for the formation of immunological memory.

How can different cell fates and cell states be reached?

As has been put forward by Buchholz and colleagues, the antigen-specific T cell compartment formed upon infection can be considered an antigen-specific tissue [9]. Akin to the development of other tissues, a population of progenitor cells (i.e., naïve T cells) is able to give rise to multiple different cell types (i.e., different effector and memory cells). Furthermore, although formal evidence for this is still lacking, part of the cells constituting this tissue are thought to possess a stem cell-like capacity to renew, as based on the ability of the antigen-specific T cell pool to expand repeatedly upon recurrent infections. The view of T cells as forming an antigen-specific tissue brings up two central questions that have also been at the center of stem cell research in other areas [10]. (i) How are different cellular subsets formed from – at least superficially – homogeneous naïve T cells? Conceptually, cellular diversification within the T cell compartment could either arise at the level of the individual naïve T lymphocyte (i.e., with each naïve T cell yielding similar proportions of SLECs and MPECs) or at the level of a population of naïve T lymphocytes (i.e., with some naïve T cells preferably or exclusively yielding SLECs and others mostly yielding MPECs as output). In other words, the central question here is whether the output of each single cell mirrors the diversity of the entire antigen-specific T cell pool (which we will refer to as single cell asymmetry), or whether the population response reflects the average of disparate individual cell behaviors (something we will refer to as population asymmetry). (ii) Do either cell-intrinsic or cell-extrinsic signals dictate the fate of the daughter cells that are formed upon T cell proliferation? In other words, does a T cell decide itself which differentiation pathway its daughters will follow, or does the environment impose a certain differentiation pathway?

The combination of possible diversification at either the single-cell or the population level with either cell-intrinsic or cell-extrinsic mechanisms yields the four fundamental scenarios for T cell diversification (Figure 1).

Although the analogy with stem cell differentiation is helpful to illustrate basic mechanisms of T lymphocyte fate determination, it is also important to realize that an antigen-specific T cell pool is a rather unusual tissue. Specifically, in other adult tissues, organ size and cellular composition are stable features, requiring relatively little flexibility with respect to kinetics of cellular turnover and differentiation. Furthermore, in the case of damage of these tissues, a default regenerative response may well suffice. By contrast, T cell expansion and differentiation necessarily have to retain a high degree of flexibility, to be able to respond to the specific requirements of a particular infection. Therefore, from a theoretical point of view, an entirely cell-autonomous regulation of T cell fates appears difficult to reconcile with the flexibility required. As a second noteworthy aspect of the antigen-specific T cell

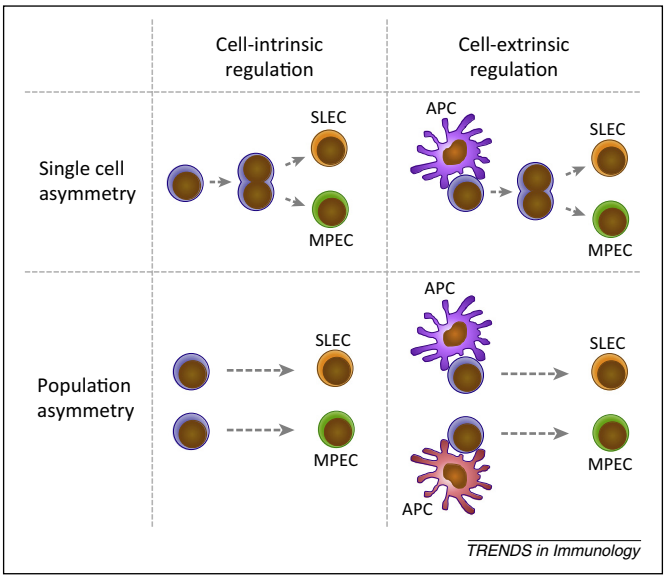

Figure 1. Matrix depicting the two axes of fate-directing mechanisms in the differentiation of naive T lymphocytes (blue) into SLECs (orange) and MPECs (green). As indicated within the figure, both single cell asymmetry and population asymmetry may potentially result from internal variability in gene expression levels, or from external, niche-imposed signals. Note that in this figure, the most extreme version of population diversification is depicted, in which individual cells only produce either SLEC or MPEC. Adapted from [10]. Abbreviations: MPEC, memory precursor effector cell; SLEC, short-lived effector cell.

tissue, the ability of antigen-specific T cells to enter distinct anatomical sites exposes them to highly different cellular environments, thereby providing ample opportunities for niche-derived signals to influence T cell fate. Based on these two theoretical considerations, it seems plausible that external, niche-directed signals play at least some role in T cell diversification. What are the data?

How to measure T cell fate acquisition?

The classical strategy to analyze T cell differentiation has been the analysis of populations of antigen-specific T cells at various times after infection. These studies have been essential to demonstrate that different T cell phenotypes dominate distinct phases of the immune response. Although these analyses have been informative to describe T cell-based immune responses at the T cell population level – the process that evolutionary pressure has acted upon – they do not reveal how these antigen-specific populations are formed. For example, are T cells at a time point B descendants from all or from just a small fraction of the cells present at an earlier time point A? Are T cells present at two different locations derived from a single naïve T cell? Are T cells with functional or phenotypic properties α or β derived from the same naïve T cells as T cells with property γ? To answer such questions that address how the antigen-specific T cell tissue is formed, it is necessary to use technologies allowing the tracking of individual cells and their progeny over time, at different anatomical locations or across diverse phenotypic subsets.

The earliest and most widely used method for single cell tracing has been time-lapse microscopy. The beauty of time-lapse imaging lies in its ability to generate complete ontological trees upon proliferation of individual cells. Furthermore, the combination of time-lapse imaging with