Division of Labor with a Workforce of One: Challenges in Specifying Effector and Memory T Cell Fate

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for the same DNA binding sequence at a chromosomal site or for an established chromatin (histone code) domain may also determine the transcriptional activity of a regulated gene. In these cases, fluctuating concentrations of nuclear factors with disparate activities during development can allow for a transcriptional flexibility that would obey mass action rules by establishing working equilibria between activating and silencing components (*12*). Such equilibria may be modified not only by varying the production of any particular factor but also by regulating the rate of their synthesis, stability, and degradation, as well as by sequestering them in different nuclear compartments. In the latter case, gene activity could be determined by moving genes into nuclear compartments where different types of regulators predominate. Indeed, the positioning of gene loci within nucleus subdomains has emerged as a potentially important determinant of gene activity (*27*, *30*, *31*). Genes associated with heterochromatic regions of the nucleus (perinuclear, centromeric clusters) seem to be silent. So far, the association is correlative, and it is unclear whether the silencing precedes or is the result of this localization. Better characterization (composition and dynamics) of such active or silencing regions will require the identification of molecules responsible (i) for setting up the environment in these domains and (ii) for the movement of genes from one region of the nucleus to another. Improvements in resolution and specificity of the tools needed for the identification and visualization of these components will be one of the most formidable technological challenges in the forthcoming years.

Concluding Remarks

The hematopoietic system, in which cell lineage choices are well characterized and a substantial number of transcription regulators of cell fate and their targets have been identified, provides an excellent paradigm to study the mechanisms that underlie lineage progression and plasticity. Initial steps in such studies are already identifying epigenetic states by which lineage priming and plasticity are achieved and are suggesting that the three discrete states of chromatin may be achieved by different mechanisms at different stages in the hematopoietic lineage. The ability to use alternative mechanisms at multiple steps during differentiation makes the hematopoietic system an important contributor to future research on epigenetic models of gene regulation in normal development and disease.

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PERSPECTIVE

Division of Labor with a Workforce of One: Challenges in Specifying Effector and Memory T Cell Fate

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In the course of the immune response against microbes, naïve T cells proliferate and generate varied classes of effector cells, as well as memory cells with distinct properties and functions. Owing to recent technological advances, some of the most imposing questions regarding effector and memory T cell differentiation are now becoming experimentally soluble: How many classes of antigen-specific T cells exist, and how malleable are they in their fate and in their functional state? How might a spectrum of cell fates be imparted to the clonal descendants of a single lymphocyte? Where, when, and how does pathogen-associated information refine the instruction, selection, and direction of newly activated T cells as they perform their tasks in different locations and times? Some surprising new glimpses ahead on these subjects and other yet-unanswered questions are discussed.

Specific immunity adapts to the threat of pathogen attack with vigorous clonal expansion of a selected lymphocyte whose antigen receptor binds microbial peptide in the context of self major histocompatibility molecules. The culmination of specific immunity is the generation of effector cells that are responsible for acute elimination of the pathogen and memory cells that patrol their various tissue domains in search of evidence of re-attack.

Heterogeneity is a hallmark of antigen-specific T cells. $CD4^+$ T cells make effector choices to become T helper cell 1 (T_H1), T_H2, or T_H17 cells and might likewise choose to become antigen-specific regulatory cells (*1*–*3*). In addition to choice of cytokine repertoire, effector $CD4^+$ T cells exhibit diversity in homing, such as migration to peripheral nonlymphoid tissue versus transit to lymph node follicles to promote B cell help (*4*). Heterogeneity of $CD8^+$ T cell effector gene expression has been described (*5*), although it is not clear whether this represents physiologically distinct cell fates or simply fluctuation in activation state. Memory T cells are heterogeneous, with central memory cells that patrol secondary lymphoid tissues, recapitulating the surveillance of their naïve progenitor, and effector memory cells that act as sentinels standing guard at frontline barriers (*6*).

Although the role and function of effector and memory subsets in protection or pathology and the nature of polarizing signals required for their differentiation are becoming increasingly clear, there are still outstanding questions that need to be addressed that relate to the mechanism of T cell fate specification. Many of these questions deal with fundamental uncertainties that are common to many areas of blood differentiation, such as the extent of fate diversity, the ontogeny and lineage relationship between opposing and kindred fates, and the degree of natural and therapeutic plasticity at different stages of differentiation.

"One Cell, One Fate" Versus "One Cell, Multiple Fates"

Signaling and transcription during T cell activation have traditionally been viewed as a uniform process. Any given naïve precursor cell could be

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signaled in a unique manner, giving rise to homogeneous progeny. In this "one cell, one fate" model, heterogeneity in cell fate can be accommodated by recruitment of several clones, leading to diversity at the population level (Fig. 1). This might occur, for instance, in different anatomic zones or at differing time points during the immune response if stimulatory conditions change due to decreased availability of antigen, decline in antigen-presenting cell function, and increased competition among antigen-specific T cells (*7*).

Clonal selection during an immune response is, of necessity, intimately associated with cell division. The possibility exists, therefore, that one cell could be signaled in such a way that it gives rise to daughter cells with identical antigen receptors that adopt different fates. In a "one cell, multiple fates" model (Fig. 1), the challenge is to determine the mechanism that generates heterogeneous progeny and whether the process is deterministic or stochastic. In any model of heterogeneity, moreover, it is important to determine whether an observed change in a cell or its progeny relates to a transition in fate (inheritable differences) or simply an alteration in its functional state (stable or unstable variation within a single fate). For example, T_H1 cells that secrete interleukin-10 (IL-10) in a provisional manner in order to establish some control over inflammation may represent an altered state of T_H1 activation rather than a distinct cell fate (*8*).

Asymmetric Cell Division and Strength of Stimulation

A deterministic mechanism to generate heterogeneity, called asymmetric cell division, has recently been proposed as an explanation for achieving fate diversity in the daughter cells (*9*). On the basis of imaging studies, it appears that a naïve T cell has a prolonged interaction with the dendritic cell (DC) before its first division (*10*). This sustained contact at the level of the immunological synapse appears to coordinate the plane of cell division and the unequal partitioning of fate-determining proteins to daughter cells (*9*) (Fig. 1). Accordingly, the first daughter T cells could represent effector- and memory-fated daughters. The daughter proximal to the synapse may become signaled more strongly such that it adopts a terminally differentiated effector fate characteristic of effector and effector memory T cells, whereas the daughter distal to the synapse may remain in an intermediate stage of differentiation, which is characteristic of the central memory T cell lineage.

The extent and nature of naïve T cell differentiation are determined by the quantity and quality of stimulation, including concentration of antigen, costimulatory molecules, and cytokines, as well as the frequency of responding T cells and density of antigen-presenting DCs (*11*). As a function of the strength of stimulation, naïve T cells progress through hierarchical thresholds for proliferation, acquisition of responsiveness to homeostatic cytokines, and differentiation to effector cells. In the clonal burst of an immune response, the four-dimensional itinerary of daughter and granddaughter T cells has not yet been adequately chronicled. The subsequent interactions of these critical progeny with their antigen, cytokine, and chemokine environment could indeed be random. If daughters inherit differing migratory or signaling capacities, however, differences in their subsequent itinerary might be considered deterministic. Random and nonrandom differences in subsequent signaling might, therefore, be critical for the descendants of a workforce of one to reach different states of differentiation, including terminally differentiated cells as well as uncommitted and less differentiated intermediates, further embellishing a spectrum of differences among clonally related T cells (Fig. 2).

The future challenge in this regard will be to determine the subsequent contact, migratory, and signaling history of daughter and granddaughter T cells, across both the $CD4^+$ and $CD8^+$ lineages and across an array of varied immune responses. How many rounds of asymmetric or symmetric division occur? To what degree do subsequent interactions appear stereotyped or random? Where do daughter cells go after division? Will the outcome of division be different for antigens presented by DCs, B cells, and macrophages?

Effector Heterogeneity—Many New Choices

Recent years have seen remarkable progress in understanding how innate immunity and other signaling processes shape the function and migration of activated T cells, but how these stimuli act on an expanding T cell clone and how much diversity is generated in immune responses remain to be determined. Indeed, with improved methods to analyze phenotype and function of effector T cells, it has become clear that the effector cells are heterogeneous in terms of their surface phenotype, cytokine production, and homing potential. Thus, in addition to the classical T_H1, T_H2, and T_H17 cells (*3*, *12*), other effector cells have been identified. These include follicular T_H cells that produce IL-10 and IL-21 (*4*), T_H0 cells producing interferon-γ (IFN-γ) and IL-4, as well as T_H cells producing both IFN-γ and IL-10 (*8*) or IFN-γ and IL-17 (*13*). Further heterogeneity is detected at the level of homing receptors with distinct subsets of central memory and effector memory cells (*6*). Although some of these properties may be related to the state of activation, most appear to define distinct T cell fates that are stable upon clonal expansion in vitro (*14*).

Many of the challenges facing our understanding of effector differentiation relate to questions of plasticity and lineage relationships. Traditionally, $CD4^+$ T cell effector fate was thought of as a binary choice between opposite fates. With increasing recognition that there are more than two $CD4^+$ effector fates (*3*, *12*), a challenge will be to understand how cells can make decisions when confronted by multiple choices. In view of the findings that mature $CD4^+$ effector fates are not adopted until after cell division (*15*), that $CD4^+$ T cells also exhibit asymmetric division (*9*), and that $CD4^+$ T cells may reengage with DCs after they have divided (*16*), it is tempting to speculate that a $CD4^+$ T cell might not make exclusive choices initially. Instead, it might generate an array of lineage-committed progenitors through limited rounds of asymmetric division. For example, the receptor for IFN-γ is polarized at the immunological synapse of $CD4^+$ T cells activated in vitro and in vivo (*9*, *17*). Because this asymmetry persists until

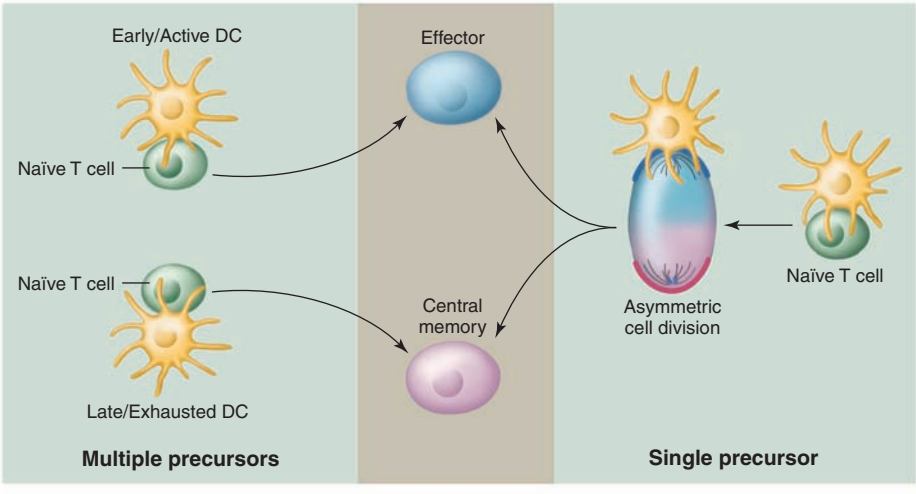

Fig. 1. Alternative models for generating heterogeneity of T cell fate during the immune response. In a "a one cell, one fate" model (left), two naïve T cells receive distinct signals from independent encounters with DCs having different states of maturation. The alternative signaling will independently elicit effector and central memory differentiation (center). Alternatively, in a "one cell, two fates" model (right), a single naïve T cell might undergo an asymmetric cell division, resulting in daughter cells that will give rise to different fates (center).

mitosis, it could result in the first $CD4^+$ daughter T cells becoming more and less T_H1-prone. With subsequent division, daughters could further diversify as T_H2-, T_H17-, or adaptive regulatory T cell (T_{reg})–prone, as well as precursors of the central memory lineage. Selective pressures mediated by the pathogen-related signals, such as presence or absence of IL-12 or IL-23, could then determine the relative outgrowth and suppression of the various lineages, resulting in a polarized outcome. Whether the initial progeny are indeed partially committed down different paths or are all equally malleable intermediates has not yet been adequately evaluated.

How many generations of daughters recontact antigen-presenting cells and whether both initial daughters have an equal likelihood of reencountering antigen in the site of primary immunization or in peripheral tissues are not yet known. It is likely that such reencounters might influence the capacity of daughter cells to further differentiate by acquiring additional functions. At which cell division number, or after how much signaling, and in what anatomic location differentiating progeny lose their plasticity and become more epigenetically fixed in their fate are unresolved issues with important therapeutic implications for diverting burgeoning and established immune responses. Another critical challenge in effector differentiation is to better understand the role and regulation of antigen-specific regulatory cells in policing their effector kindred (*2*). Whether variant effector T cells that coexpress regulatory cytokines (*8*) and antigen-specific adaptive T_{reg}'s expressing FoxP3 (*12*, *18*) will play roles in a physiological or therapeutic setting across a wide spectrum of immune responses has not been fully evaluated.

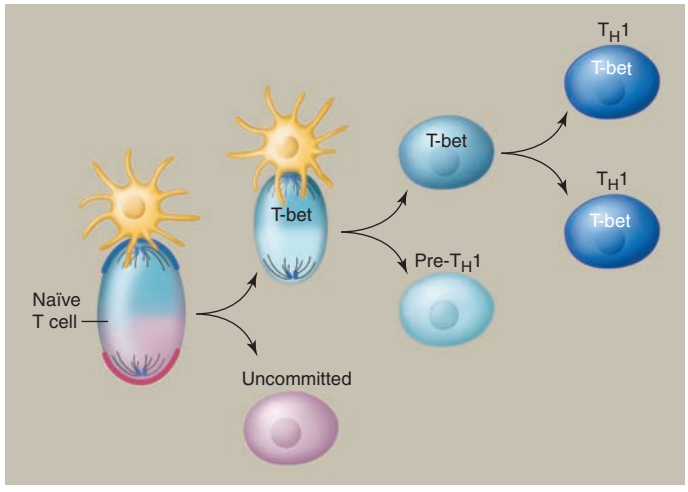

Fig. 2. Hypothetical model for generating a spectrum of differentiation among the descendants of a single clonally selected T cell. Daughter cells could encounter stochastic variation in exposure to antigen or cytokine signals, as well as deterministic differences such as unequal inheritance of fate-imposing or migratory signals. Together with further asymmetric cell division, these random and nonrandom differences in strength of stimulation could yield a diverse clonal burst, including but not limited to terminally differentiated effector progeny (T-bet–expressing T_H1 cells, in this case) as well as progeny with uncommitted and intermediate states of differentiation.

Memory Heterogeneity and "Stemness"

The ontogeny of memory T cells is controversial, with some studies suggesting a progressive development of memory cells from effectors and others suggesting an early bifurcation between commitment to the memory and effector lineages (*6*, *7*, *9*, *19*, *20*). An asymmetric first T cell division provides a potential mechanism for simultaneous generation of effector and memory daughter cells, with the proximal daughter poised to receive more antigen and cytokine signals compared to the distal cell, which can retain stem cell–like plasticity for renewal and further differentiation at a later time (*9*) (Fig. 1).

If the first distal daughter is the precursor of a central memory–like cell, then it remains to be determined what are the precursors of effector memory T cells. Are effector memory T cells simply long-lived effector cells that escaped clonal deletion, or are they continually repopulated from a stem cell–like central memory cells? Whereas the first mechanism would ensure that the spectrum of functions generated in the primary response are maintained in memory cells, the second mechanism is consistent with the finding that effector memory cells have poor reconstitution potential and that a central memory T cell can differentiate to effector cells in response to homeostatic cytokines. Because a central memory stem cell periodically engages in intercellular communication to receive membrane-bound IL-15 signaling during homeostasis, it is possible that a nonantigen-driven asymmetric division might result in a self-renewing central memory stem cell daughter and a terminally differentiated effector memory daughter.

Technical Challenges—Imaging and Modeling a Workforce of One

Modeling the immune response in vivo has relied heavily on the transfer of relatively high numbers of transgenic T cells expressing an antigen receptor of known specificity. It is increasingly apparent, however, that the behavior of 1 million transferred cells might be quite distinct from the behavior of a single cell in isolation (*19*, *21*, *22*). A high frequency of responding cells might result in strong competition, decreasing the duration and strength of the stimulus received and perhaps the likelihood of asymmetric division, which might ultimately lead to incomplete differentiation. The in vivo monitoring of a physiological immune response would require methods that allow tracking of endogenous antigen-specific T cells and, eventually, a single naïve T cell. Such minute numbers of cells, however, would make it difficult to image cell-cell interactions and clonal dynamics in situ. Given that the ability of a responding T cell to maintain contact is inversely proportional to the number of responding cells (*21*), it might be extrapolated that asymmetric division would be an invariant feature of clonal bursts with only small numbers of responding cells.

As mentioned above, a major challenge facing the field is the ability to chronicle a clonal burst adequately in vivo. Reagents and model systems to trace early events developing in situ will be critical for this task. Imaging approaches that can reveal cell fates using reporters of key transcription factor or effector molecule expression will undoubtedly be useful to this end (*13*). Being able to distinguish parent from daughter and granddaughter, as well as which are descendants of proximal and distal cells, will be important to refine a fate map of different immune responses. Reagents that can report the maturation status of DCs and local cytokine gradients may also be necessary to understand the role of the T cell's environment in promoting variance in the fate of the activated cells (*23*). The field is now faced with the exciting, yet daunting, challenge of unveiling the instructions, identity, and agenda of the cellular descendants of a single T cell called to battle.

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PERSPECTIVES

Private Lives: Reflections and Challenges in Understanding the Cell Biology of the Immune System

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The immune system comprises a variety of cell types whose activities must be carefully regulated to act as a coherent unit for the purpose of host defense. To perform their autonomous functions, immune cells must rely on the same basic organizational features that apply to all cells, although immune cells often exhibit remarkable degrees of specialization and adaptation. The study of these specializations has lagged behind advances in understanding the immune response and cell biology individually. As a result, there are great opportunities, but also great challenges, for new conceptual discoveries by taking a more cell-biological approach to probing the function of the immune system.

Immunology and cell biology share at least one profound common origin. Around the turn of the 19th century, Elie Metchnikoff discovered "innate immunity" by demonstrating the ability of phagocytes to detect, engulf, and kill invading microbes. His definition of this fundamental principle of immunology was wholly enabled by paying close attention to the cell biology of how phagocytes worked. Metchnikoff's attentiveness also provided some of the first fundamental principles in cell biology, including the discovery of endocytosis, the function of lysosomes, and the ability of cells to produce cytotoxic compounds. Despite this auspicious beginning, immunology and cell biology gradually drifted apart. Perhaps because of the emerging complexity of each field, immunologists became less interested in how the cells they study actually work, whereas cell biologists (at least molecular cell biologists) avoided problems involving more than one or two cells. Yet today, like Amanda and Elyot, the divorced couple in Noel Coward's play *Private Lives*, immunology and cell biology now find themselves with new spouses in adjacent hotel rooms, realizing that there had been something wonderful in their previous relationship.

In all complex problems, understanding the mechanism provides the key to understanding the problem itself, even if this relationship is hidden by a preoccupation with the problem. In immunology, this key was long ago demonstrated by the application of molecular biology to unravel how immune cells generate the diversity required for antigen recognition by antibodies and T cells. Attention to cell-biological mechanisms has similarly produced basic insights, particularly in the areas of leukocyte diapedesis, apoptosis, and transcription. What we have learned from studying cytotoxic T lymphocyte (CTL) function has been particularly noteworthy in this regard. We have learned that cytotoxic granules are in fact modified lysosomes whose distribution and secretion are polarized to the site of target-cell recognition, increasing the directionality and thereby the selectivity of the cytotoxic payload. CTL granules polarize by interacting with microtubule-dependent motor proteins after an induced reorientation of microtubule organizing centers and thus the cell's entire microtubule network after antigen recognition (*1*). Both granule biogenesis and polarity were first described with classical cell-biological approaches and then confirmed by the analysis of mutant cells isolated from individuals with various inherited immunodeficiency syndromes (*2*). These studies demonstrated that the features of granule dynamics defined for CTLs apply to other secretory cell types (such as melanocytes). Other surprises await in these systems, such as the posttranscriptional regulation of cytokine production in secretory cells [such as natural killer cells (*3*)].

A variety of other critical problems in the immune response could also be understood at a similar level of cell-biological resolution, revealing basic new information about both immunology and cell biology. We know remarkably little about the mechanisms of cytokine secretion (especially cytosolic cytokines such as interleukin-1β), how cytoplasmic scaffolds control T cell receptor signaling, how Toll-like receptor signaling is controlled in different intracellular compartments, what the immunological synapse actually does and how it works, and the mechanisms by which alterations in cell adhesion cause cellular activation or deactivation. Another central problem in immunology that has created a natural interface with cell biology, the one that we have engaged, is antigen processing and presentation. None of these are new problems. However, they have failed to capture the imagination of more than a handful of cell biologists, leaving them to immunologists, many of whom must learn the methods and criteria of cell biology on the job. The relative lack of interaction between the two communities has created a number of disconnects over the years that perhaps have made progress more difficult to achieve than it already is.

Pathways of Antigen Processing: MHCII

With the realization more than 20 years ago that major histocompatibility complex class II (MHCII) molecules bound peptides derived largely from extracellular antigens, there has been much interest in understanding the pathways and organelles involved. It was appreciated early on that an invariant chain directed newly synthesized MHCII αβ dimers to be diverted from the secretory pathway into endocytic organelles where they encountered internalized antigens (*4*, *5*). Proteases clearly degraded invariant-chain and protein antigens, with peptide loading facilitated by chaperones such as HLA-DM, and the resulting peptide-MHCII complex then proceeded from the loading site to the plasma membrane. However, the identity of the intracellular compartment(s) in which these events transpired (as well as the order of events) remained uncertain.

Initially, the issue was, in effect, ignored by collectively referring to any endocytic organelle containing MHCII as the MHCII compartment (MIIC) (*6*). This raised a problem because the endocytic pathway comprises several distinct organelles that have decidedly different functions. Worse, the term MIIC came to imply the existence of a unique compartment specific to antigen-presenting cells (APCs). This situation obscured an underlying complexity of functional importance and substituted it with a complexity (i.e., a novel organelle) that probably did not exist (*7*, *8*). MIICs were generally assumed to have the properties of late-endocytic compartments, namely because the cells most commonly used in these studies localized most of their MHCII to late endosomes and lysosomes. Yet, not all APCs accumulate MHCII in late compartments, not all antigens are processed in late compartments, and not all APCs maintain a characteristic distribution of MHCII under all conditions. This serves to illustrate the importance of understanding organelles of immune cells with

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