Nine lives: plasticity among T helper cell subsets

Richard M. Locksley

The division of labor among two types of T helper (Th) subsets, first described over 20 yr ago, has been buffeted by the discovery of new subsets and new cytokines that can be coaxed out of T cells with increasing disregard for the subset of origin. Although Th17 cells and regulatory T (T reg) cells are widely accepted subsets, and others are being proposed, their plasticity is difficult to reconcile with the definitions of Th subsets as put forth in the initial description of Th1 and Th2 cells. A deeper molecular context will be required to reconcile the ever-increasing complexity of effector T cells.

The Journal of Experimental Medicine

What's in a name?

On page 1653 of this issue, Nowak et al. (2009) address the production of IL-9 by helper T cells, a finding that is of interest because of recent studies that found TGF-β could induce the differentiation of "Th9" cells with a unique pattern of cytokine expression and a transcription factor profile that could not be pigeonholed into the framework of the Th1, Th2, Th17, or T reg cell subsets (Dardalhon et al., 2008; Veldhoen et al., 2008). The role of TGF-β in invoking IL-9 production from T cells was originally reported by Schmitt et al. (1994), who also noted that IL-4 could potentiate and IFN-γ could inhibit the effect. TGF-β regulates the differentiation of both T reg and Th17 cells and, in this issue, Nowak et al. (2009) demonstrate IL-9 production by Th cells primed in the appropriate manner and purified using reporters for FoxP3 or IL-17F (canonical signatures for T reg and Th17 cells, respectively). This suggests that IL-9 production tracks with priming or activation in the presence of TGF-β rather than with a defined Th subset. The authors go on to use knockout and chimeric mice to show that the IL-9 made by Th17 cells is proinflammatory in a standard myelin oligodendrocyte glycoprotein (MOG) peptide-induced model of experimental autoimmune encephalomyelitis (EAE). This result adds more complexity to the issue, as members of this group had previously implicated a role for IL-9 produced by T reg cells in mediating graft tolerance by a mast cell–dependent process (Lu et al., 2006). Thus, IL-9 could be either inflammatory or regulatory, depending on the context and the source of the producing cells. In the hands of another group, IL-9 produced by TGF-β–primed Th17 cells enhanced the suppressive effects of T reg cells, resulting in an exacerbation of EAE (although diseas was induced using a different dose of antigen) in the absence of IL-9 (Elyaman et al., 2009). The precise reason for the discrepancies remains unclear. Everybody seems to agree that TGF-β can induce IL-9 production from helper T cells, but the biology beyond that will require further investigation to clarify the details.

So where does this leave us with "Th9" cells? In the earlier studies, in vitro priming using anti-CD3 and irradiated APCs or anti-CD3 and anti-CD28 was used to generate FoxP3-negative, IL-9–producing (and IL-10–producing) cells as assessed by intracellular cytokine detection after restimulation with PMA and ionomycin; conditions much like those used in the Nowak et al. (2009) paper. But are the resulting cells truly a unique Th subset?

Studies in the mid-1990s demonstrated that cells primed once or twice were not stable when subsequently stimulated under different conditions, and they displayed much more plasticity than did cell lines restimulated multiple times under polarizing conditions (Murphy et al., 1996). Indeed, the original definition of Th subsets was based on stable cytokine patterns produced by several mouse clones that were established from mice immunized in vivo and then cloned and maintained in vitro, and these cells provided the basis for the Th1 and Th2 designations (Mosmann et al., 1986). The cell's cytokine patterns remained stable whether assessed after stimulation with Concanavalin A, antigen and APCs, or allogeneic APCs over periods up to 18 mo. No subsequently defined Th subsets, including T reg and Th17 cells, have stood up to such a rigorous examination. Rather than discarding the concept of Th subsets outside of the original Th1 and Th2 cells, however, it may be useful to consider the expansion of knowledge regarding intrinsic and extrinsic factors that come to bear on the ultimate elaboration of helper T cell cytokines.

Inside the box

The identification of stable Th cell lines enabled discovery of the molecular bases for the disparate patterns of cytokine expression and uncovered the epigenetic underpinnings of Th cell differentiation. As recently reviewed (Wilson et al., 2009), T cells integrate signals from dendritic cells and cytokines that together establish a network of DNA-binding factors to initiate feed-forward systems that epigenetically modify chromatin such that patterns of gene expression come

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The Rockefeller University Press $30.00
J. Exp. Med. Vol. 206 No. 8 1643-1646
www.jem.org/cgi/doi/10.1084/jem.20091442

to be recapitulated in a heritable way. Breaking the epigenetic code facilitated recognition of unique modifications associated not only with active or silenced genes, but also with "poised" genes that could be activated when signaled, as well as genes carrying "bivalent'" marks that were capable of further modification (be it activation or silencing) upon subsequent exposure to the appropriate signals. In this way, differentiated cells become heritably restricted in their phenotypes, yet maintain some flexibility to tailor responses to changes in the environment, much as hematopoietic precursors traverse "bistable" states on their way to fixed cell lineages (Laslo et al., 2006) (Fig. 1).

Recent studies have interrogated CD4 and CD8 T cells by combining gene expression profiling with ChIP-Seq and high-throughput sequencing technologies to investigate the chromatin state in resting and effector T cells, providing a first molecular look at the status of cytokine genes accompanying differentiation (Araki et al., 2009; Wei et al., 2009). The surprising finding was the retention of bivalent marks at transcription factor genes previously identified as "master regulators," thereby predicting a large degree of flexibility that could be revealed by subjecting cells to alternate conditions that activate the bivalent state. The presence of bivalent marks at *Tbx21* and *Gata3* in Th17 cells, at *Gata3* in Th1 cells, at *Tbx21* in Th2 cells, and at *Tbx21*, *Gata3*, and *Rorc* in T reg cells suggests the potential for substantial reversibility of function, which has now been confirmed experimentally (Lee et al., 2009; Wei et al., 2009). Notably,

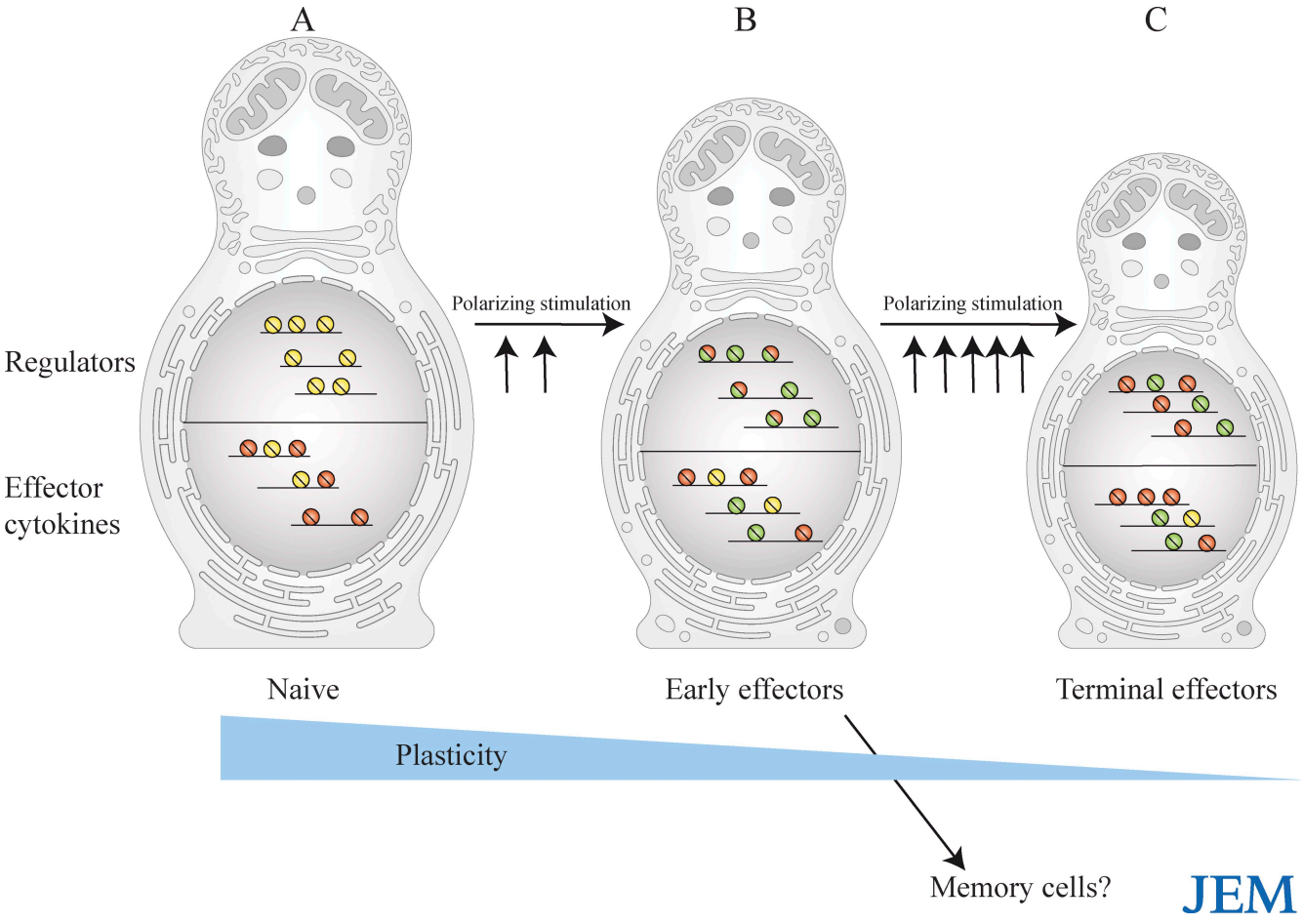

Figure 1. Epigenetic modifications accompany progressive T helper cell differentiation. (A) Naive T cells have master regulators (top) in receptive "poised" states (yellow balls) and effector cytokines (bottom) in epigenetically silenced chromatin (red balls), although some cytokine genes are poised for rapid expression after TCR stimulation. (B) After one or two stimulations under polarizing conditions, daughter cells express similar cytokines from activated loci (green), whereas other cytokines become silenced (red) or maintain a poised state awaiting stimulation. Some master regulators, however, remain bivalent (red and green), sustaining flexibility in the cells that allows redirection of cytokine expression if the inflammatory milieu changes. (C) With continued polarizing stimulations, master regulators become silenced (red) or activated (green), leaving cells with an essentially fixed repertoire of cytokine effectors (bottom) caused by the "loss" of genetic space. Memory cells, which arise early after initial antigen stimulation, would be predicted to maintain more bivalent states at master regulators, thus leaving a more flexible cytokine repertoire in memory cells as compared with terminal effector cells.

however, these studies generally used two rounds of polarization in vitro, and are subject to the caveats noted above. Such findings need to be extended to analyses of highly polarized cells generated in vivo and then cloned and sustained by the methods used to generate the initial Th1 and Th2 cells to assess whether further restrictions of lineage regulators occurs as cells are pushed into increasingly more confined genetic space.

Outside the box

Impinging upon this variegated nuclear architecture of the genome is the complex environment of cytokines and cell surface molecules that together constitute the system for modifying gene expression upon TCR ligation. It should not be surprising that some constellation of signals could activate the right network of transcription factors such that activation of unanticipated genes, potentially those carrying bivalent marks, will occur, thus achieving new states of cytokine expression. If some of those genes encode key regulators that can themselves affect chromatin architecture, reversibility in phenotype will invariably occur. This is particularly true if coupled with signals that activate cell division, a process that favors the establishment of new marks on the daughter chromatin strands. After all, only four transcription factors are required (including c-Myc to promote entrance into cell cycle) to convert fibroblasts into pluripotent stem cells, although a more thorough understanding of how to reprogram DNA and histone modifications will be necessary to improve the efficiency of the process (for review see Yamanaka, 2009).

Understanding the pathways that activate genes with bivalent marks will increase our understanding of the regulatory networks involved in T cells, and perhaps allow the strategic targeting of key pathways to redirect immunity in therapeutic ways. Based on the reports of "Th9" cells, we might predict that the IL-9 gene will carry a "poised" or bivalent mark in most activated T cells, but that this mark will increasingly tilt toward the activated state in the presence of TGF-β and toward the silenced configuration in the presence of IFN-γ. Similarly, IL-10 can be induced from a wide variety of Th subsets under different conditions, but IL-27 might provide an entrance into the network that converts this cytokine gene to the fully activated state in certain subsets (Stumhofer et al., 2007). A more complete analysis of the bivalency epigenetic map in Th cells as they undergo successive rounds of division may reveal the critical paths that sequentially close down as cells terminally differentiate, as well as the key shortcuts that remain open to enable some cells to escape their terminal fates and reset their effector functions. Such approaches may also help define important transition states, such as those that create T follicular helper cells, that have been related to Th subsets through their cytokine expression status (King and Mohrs, 2009; Reinhardt et al., 2009; Zaretsky et al., 2009).

Russian dolls

Helper T cell differentiation can be compared with nested Russian dolls, where each daughter represents a subset of the precursor from which it arose. Although descended from the same genetic space, the environment will compress the accessible space sequentially, leaving each daughter less flexible than its predecessor (Fig. 1). Thus far, descriptions of Th subsets typically rely on once- or twice-primed cells, restimulation assays, whole population approaches (PCR, ELISAs, etc., that by necessity rely on the sum collected from many cells), or artificially in vitro activated cells—all done for various technical and financial reasons. Functional genomic analysis of more deeply nested dolls will be necessary before we can put the seminal observations of Mosmann et al. (1986) into the proper molecular context to arrange the increasing array of putative Th subsets into their proper hierarchical space. With single-cell genetic, sequence, and proteomic analysis getting closer and closer to reality, it will be only a matter of time until we find out whether truly differentiated Th cells really have nine lives or nine cells have one.

The author acknowledges stimulating conversations with members of his laboratory and support from Howard Hughes Medical Institute and the National Institutes of Health.

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