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Of Origins and Pedigrees: Lineage Tracing of Dendritic Cells

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http://dx.doi.org/10.1016/j.cell.2013.07.044

Understanding the ontogeny of distinct hematopoietic cell types remains a challenge. In this issue, Schraml et al. contribute to unraveling the complexity of a central component of the mononuclear phagocyte system by using a new in vivo approach to trace the progeny of common dendritic cell precursors.

"It was exciting when dendritic cells appeared first as novel cells, and it remains exciting that these cells represent a novel force in medicine" (Steinman, 2012). After the original discovery of dendritic cells (DCs) by Steinman and Cohn in 1973, mainstream immunologists took several decades to fully recognize the pivotal role of these myeloid leukocytes. Today, DCs are well established as central instigators and regulators of both innate and adaptive immune responses and have been used in preclinical and clinical settings to treat inflammatory diseases, alloimmunity, infections, and cancer. Consequently, in 2011, Ralph Steinman was awarded the Nobel Prize in Physiology or Medicine for his discovery.

Strategically positioned in the skin, on mucosal surfaces, and in other tissues throughout the body, DCs are effective sensors and phagocytic collectors of antigenic material from a variety of sources, including pathogens and cancer cells; when DCs acquire antigen in a peripheral tissue, they migrate to lymphoid organs, where they function as potent activators of antigen-specific lymphocytes (Merad et al., 2013). However, not all DCs are created equal; a growing number of subsets have been identified in both mice and humans that express distinct phenotypic markers and play specialized roles in the immune system. A better understanding of the origins of these subsets and the rules that determine their production and function could not only generate better insights in the intricacies of immune regulation but may also reveal novel avenues for therapeutic or prophylactic immune modulation and vaccine development; however, progress in this area has been slow due to inherent limitations in hitherto available biological tools (Figure 1A).

In this issue of *Cell*, Schraml et al. (2013) report an in vivo lineage-tracing approach that refines our understanding of DC subset ontogeny. The authors employed an in vivo genetic tagging strategy that specifically marks common DC precursors and all of their progeny by constitutive expression of enhanced yellow fluorescent protein (EYFP). This strategy not only confirms and solidifies previously held notions of the origin of several DC subsets but also yields unexpected discoveries, namely the identification of common DC precursor-derived leukocytes in the small intestine and kidney. These DC subsets were previously believed to be of monocyte origin because earlier lineage-tracing studies were unable to pinpoint their origin due to technical limitations.

Historically, new cell types have been defined based on morphology, surface phenotype, and/or function, and it has

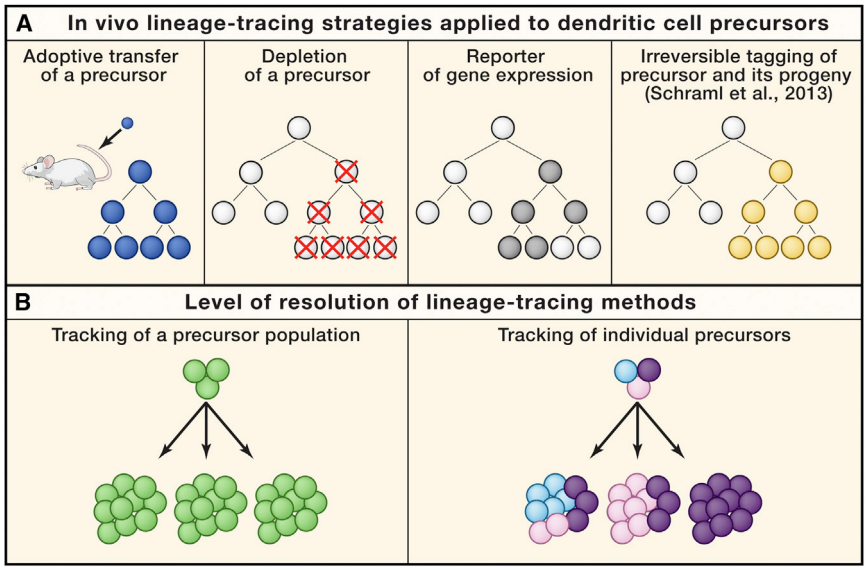

Figure 1. In Vivo Lineage-Tracing Strategies and Their Level of Resolution

(A) Different lineage-tracing strategies have been employed to investigate DC ontogeny. A common approach involves the adoptive transfer of a purified precursor population (far left). A second method makes use of selective depletion of a precursor population (middle left). A third strategy relies on the detection of a fluorescent reporter to identify cells that express a specific gene, such as a transcription factor (middle right). The new approach by Schraml et al. (2013) enables irreversible tagging of common dendritic cell precursors and their progeny (far right).

(B) Example of population-based versus single-cell lineage-tracking strategies. The top row shows two phenotypically homogeneous precursor populations that give rise to three distinctly differentiated progeny (bottom row). Unlike population-based tracking, in which all progenitors express a single tag, tagging of individual progenitors with unique labels allows the detection and quantification of heterogeneity in differentiation potential between individual precursors.

been assumed that similarities in these characteristics are indicative of a common origin. Owing to recent technical advances, many investigators have shifted their focus to analysis of transcription factor expression profiles. However, morphologic, phenotypic, functional, and gene expression characteristics of migratory leukocytes, particularly DCs, can all be influenced by environmental stimuli such as tissue-specific homeostatic and/or inflammation-induced signals, so similarities in these parameters may not always reflect descent from a common ancestor.

To faithfully track the progeny of a selected precursor, several lineage-tracing techniques have been developed (Kretzschmar and Watt, 2012), including a number of strategies that have been applied in the DC field (Figure 1A). A common approach involves adoptive transfer of purified precursors into (often irradiated) mice, whereby the transferred cells and their progeny carry a heritable marker to distinguish them from host cells. However, it is difficult to ascertain whether the transferred progenitors can occupy the same anatomic niches and access the same differentiation and survival signals in the recipient that they encountered in the donor; this raises uncertainty as to whether the transferred cells' differentiation potential truly reflects their natural state.

Another technique is based on the selective depletion of defined precursors in vivo. Subset-specific depletion can be achieved in mice by inserting a complementary DNA (cDNA) for the diphtheria toxin receptor (DTR) in a defined gene, such as a transcription factor, that is uniquely expressed in a cell type of interest. Systemic treatment of the mutant animals with diphtheria toxin (DT) can selectively eliminate the DTR-expressing cells, an approach that was recently applied to DC precursors (Meredith et al., 2012). Provided that all progenitors of interest are depleted, this strategy faithfully establishes ontogeny of the absent cell type. However, it is challenging to identify new lineage members, as the absence of a cell type will only be noted if it was under investigation in the first place.

Yet another technique involves tracking of DCs in so-called "reporter mice," whereby a suitable genetically encoded tag, such as a fluorescent protein, is placed under control of a lineage-specific promoter (Satpathy et al., 2012). Any cell that expresses the reporter gene above the detection threshold can be identified. Provided the activity of the chosen promoter is faithfully restricted to a particular lineage, essentially all lineage members can be identified. However, any progeny in which the promoter becomes inactive (e.g., in response to environmental changes) may be missed.

To overcome the limitations of these traditional lineage-tracing strategies, Schraml et al. (2013) generate a mouse model in which common DC precursors selectively express a bacterial enzyme, Cre recombinase, which excises a "stop" signal in a constitutively active genetic locus, thus enabling permanent expression of EYFP from that locus. Because the Cre-dependent activation of EYFP expression is irreversible, the fluorescent signal marks not only precursors but also all of their progeny, irrespective of any subsequent changes in transcription profile or phenotype. Illustrating the power of this approach, the authors discover two EYFP-expressing leukocyte subsets in the small intestine and kidney that had not been previously assigned to the DC lineage, thereby refuting the long-held view that these subsets originated from monocytes.

Despite the impressive sophistication of this and other hematopoietic lineage-tracing techniques, further methodological refinements are conceivable in years to come. For example, large-scale expression profiling may yield further highly specific tools for in vivo tagging of various progenitors, which could be combined with inducible promoter systems to enhance temporal resolution and tagging specificity. Furthermore, it should be feasible to improve lineage-tracing techniques to provide single-cell resolution of precursor-progeny relationships. So far, most fate-mapping studies in the DC lineage have tracked the progeny of precursor populations rather than individual precursor cells (Figure 1B). Emphasizing the importance of single-cell tracking,

recent studies have demonstrated that individual phenotypically homogenous hematopoietic progenitors can have highly heterogeneous fates (Lu et al., 2011; Naik et al., 2013; Buchholz et al., 2013; Gerlach et al., 2013). In vivo lineage tracing of single hematopoietic cells so far has only been performed in adoptive transfer settings either by transfer of genetically tagged single cells or of cell populations composed of individual cells that each carry a unique and heritable label, such as a congenic marker, fluorescent protein, or DNA barcode (that is, a random noncoding DNA sequence).

Looking to the future, the combination of specific in vivo tagging technologies with single-cell resolution strategies may ultimately lead to a deeper understanding of the origin, pedigree, and function of many cell types, including DC subsets. Thus, 40 years after the initial discovery of DCs, these multifaceted cells remain an important and exciting object of investigation.

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