DNGR-ing the dendritic cell lineage

Andreas Schlitzer & Florent Ginhoux

Dendritic cells (DCs) are a group of functionally specialized antigen-sensing and antigen-presenting cells that initiate and orchestrate immunity. DCs are uniquely equipped to recognize pathogens, vaccines and self-antigens, and to instruct simultaneously the type, magnitude and specificity of immune responses. They are remarkably heterogeneous, and identifying different subsets on the basis of phenotypic and functional characteristics has proven challenging. In a recent issue of *Cell*, the Reis e Sousa group has overcome the existing limitations by developing the first true fate-mapping model of DC progenitors [1].

Distinct DC subsets have been traditionally identified by characteristic anatomical location, phenotype and function. The broadest division separates resident DCs in lymphoid tissue (LT) from non-lymphoid tissue (NLT) DCs that migrate to the lymph nodes through the lymphatics (Fig 1). In mice, resident LT DCs are often referred to as conventional DCs (cDCs), to distinguish them from migratory DCs and plasmacytoid DCs (pDCs), which produce vast amounts of interferon- α during viral infection. cDCs are divided into two main subsets based on phenotype: CD8 α ⁺ DCs, CD4⁺CD11b⁺ DCs and a minor CD8 α ⁻CD4⁻CD11b⁺ DC subset [2]. More recently, high expression of endothelial cell-specific adhesion molecule (ESAM) was shown to characterize CD4⁺ splenic DCs [3]. Migratory DCs are generally distinguished by mutually exclusive surface expression of the integrins CD103 and CD11b, except in the lamina propria, in which an additional subset co-expressing CD103 and CD11b can be found [2]. Importantly, inflammation induces the generation of inflammatory DCs (iDCs) with additional phenotypic and functional characteristics. However, whether iDCs have a common origin with their steady-state counterparts, or arise through alternative differentiation pathways, is unclear.

Fig 1 | Ontogeny of DC subsets in lymphoid and non-lymphoid organs revealed by fate-mapping of DNGR-1 expression. DC-restricted CDPs expressing DNGR-1 arise from the MDPs and develop into pre-DCs, which migrate out of the bone marrow and seed lymphoid and non-lymphoid tissues. There, they differentiate into CD8 α ⁺/CD103⁺ or CD11b⁺ DCs. In this model, pre-DCs and their DC progeny express YFP, whereas macrophages and monocytes are not tagged. CDP, common dendritic cell progenitor; DC, dendritic cell; DNGR-1, dendritic cell natural killer lectin group receptor 1; ESAM, endothelial cell-specific adhesion molecule; LMPP, lymphoid primed multipotent progenitor; MDP, macrophage and DC precursor; M ϕ , macrophage; pDC, plasmacytoid dendritic cell; YFP, yellow fluorescent protein.

Analyses of the growth and transcription factor requirements of the different DC subsets have helped understand their inter-relationships. CD103⁺ DCs and CD8 α ⁺ DCs are considered a distinct DC

lineage, which is dependent on the *Fms*-like tyrosine kinase 3 ligand receptor (Flt3) and the transcription factors ID2, BATF3 and IRF8, and can efficiently cross-present antigens and stimulate CD8⁺ T-cell immunity [2].

By contrast, splenic CD11b⁺ DCs are known to induce potent CD4⁺ T-cell responses, and a unique function has yet to be ascribed to NLT CD11b⁺ DCs. In fact, dissecting the relationship between NLT and LT CD11b⁺ DC populations exemplifies the challenges in understanding DCs. NLT CD11b⁺ DCs—gated as MHC-II⁺CD11c⁺CD11b⁺ cells—are heterogeneous in origin, deriving from bone marrow DC-specific progenitors and monocytes, and seem partly dependent on both Flt3 and the colony-stimulating factor 1 receptor (CSF-1R) for their differentiation [4]. Indeed, this reflected a contamination of CSF-1R-dependent monocytes and macrophages within the CD11b⁺MHC-II⁺CD11c⁺ fraction that classically define DCs [5,6]. Such observations highlight the shortcomings of defining DC sub-populations solely by phenotype. Defining DC populations by function, however, is similarly unsatisfactory, as the local microenvironment can confer convergent abilities to DCs of distinct origin in steady state and during inflammation.

An alternative approach is to define DCs as a unique haematopoietic lineage distinct from monocytes and macrophages. In fact, studies on DC ontogeny have shed light on the organization of the murine DC lineage and the relationships between DC subsets, as well as their broader relationship to monocytes and macrophages (Fig 1; [2]). The macrophage and DC precursor (MDP) first gives rise to monocytes and to the common DC precursor (CDP), which has lost monocyte and macrophage differentiation potential and only generates DCs via pre-DCs. Pre-DCs exit the bone marrow and circulate in the blood to enter tissues in which they give rise to LT and NLT DCs [4]. However, this picture has been obtained by tracking small numbers of progenies from labelled bone marrow precursors that were adoptively transferred into the blood of recipient animals. Such strategies are qualitative and vulnerable to the effects of the artificial introduction of large numbers of progenitors into a non-physiological location and an occupied niche. Conditioning regimens such as irradiation and specific DC depletion increase the yield of progeny cells, but this creates inflammation, which could affect the differentiation of the transferred cells.

The Reis e Sousa group identified DNNGR-1 (CLEC9A)—a receptor for dead cells—as a marker expressed in the DC-restricted precursors, CDPs and pre-DCs, but not in MDPs. Taking advantage of this specific expression pattern, they designed a

fate-mapping model in which the Cre recombinase is expressed under the control of the DNNGR-1 promoter. When crossed with Cre reporter mice, Cre recombination irreversibly marks DNNGR-1-expressing cells and their progeny by the constitutive expression of yellow fluorescent protein—thus, CDP progeny in both LTs and NLTs can be tracked. Tracing DNNGR-1 expression successfully identified conventional DCs as CDP-derived, but additionally revealed the CDP origin of ESAM^{lo}CD11b⁺ DCs, previously thought to derive from monocytes [3]. This model also conclusively showed that pDCs do not arise from DNNGR-1⁺ CDPs, confirming previous indications of low pDC potential of CDPs, and validating the recent identification of pDC-specific precursors [7].

Schraml and colleagues then examined DC populations of uncertain origin in non-lymphoid tissues, including the lung and small intestine. Their data support the use of CD64 (FcγR1) as a discriminative marker between DCs and macrophages in these two organs, confirming previous genetic and functional studies [5,6,8]. In addition, their model identifies a previously unknown DC population in the kidney. A minor subset of CDP-derived DCs acquires a macrophage-like phenotype, expressing CD64 and F4/80, whilst retaining the DC functional ability to process and present antigen to T cells. Such data fully validates the approach of the authors in developing this model, and highlights the importance of understanding the ontogeny of a cell rather than classifying it solely by phenotype or function.

Another highly debated question in the DC field concerns the relationships between steady-state DCs and iDCs, and their respective origins. iDCs were first observed in the spleen of mice infected with *Listeria monocytogenes* [2]. Schraml and colleagues infected their fate-mapping model mice with *Listeria* and clearly demonstrated the non-CDP origin of such iDCs. Another type of iDC with potent cross-presentation capacity—which is dependent on Flt3L and expresses CD209 and the DC-restricted transcription factor Zbtb46 [9,10]—was identified in the lymph node after lipopolysaccharide treatment [2]. It would have been interesting to know if these iDCs were derived from CDPs or from non-CDP precursors undergoing an alternative convergent DC differentiation programme during inflammation.

Finally, one intriguing observation made by the Reis e Sousa group is the incomplete and uneven recombination level

of CD11b⁺ DCs. CD103⁺/CD8α⁺ DCs reached almost 100% recombination—because they maintain DNNGR-1 expression as mature cells—whereas CD11b⁺ DCs' recombination levels were between 20 and 60%, depending on the tissue analysed. As proposed by the authors, this partial labelling of CD11b⁺ DCs—which do not express DNNGR-1 as mature cells—is probably related to the incomplete labelling of bone marrow precursors (around 20%). Such incomplete penetrance is inherent to *in vivo* labelling techniques, but the tissue-specific variation in the percentage of recombination within the CD11b compartment remains unexplained: if all CD11b⁺ DCs arise from the same progenitors and similarly do not express DNNGR-1 as mature cells, why are these tissue-specific variations in labelling observed? The incomplete and uneven labelling of CD11b⁺ DCs could reflect a contribution from DNNGR-1⁺ precursors, perhaps even DNNGR-1⁺ CDPs (see minor DNNGR-1⁺ fraction in Fig 1A). If this is the case, it suggests two possible scenarios: CDPs are either intrinsically primed with specific DC subset potential or extrinsically responding to cues from the bone marrow milieu. Considering CDPs as a homogeneous entity might mask many important characteristics that could be revealed by single-cell transcriptome analysis to delineate the qualitative differences between precursors.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Andreas Schlitz and Florent Ginhoux are at the Singapore Immunology Network (SIgN), Agency for Science, Technology and Research (A*STAR), Singapore. E-mail: florent_ginhoux@immunol.a-star.edu.sg

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