GOOD THINGS COME IN THREES

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Ectopic expression of PU.1, IRF8, and BATF3 reprograms mouse and human fibroblasts into dendritic cells. See related Research Article by Rosa *et al*.

In this issue of *Science Immunology*, Rosa *et al*. describe a strategy to reprogram fibroblasts into dendritic cells (DCs) (*1*). DCs play a central role in shaping adaptive immune responses and in the generation of immune memory. Upon activation, DCs upregulate costimulatory molecules and chemokine receptors that allow their migration to draining lymph nodes (migratory DC), where they interact with antigen-specific T cells. It is now appreciated that DCs are functionally heterogeneous. Broadly, DCs have been classified into plasmacytoid DCs (pDCs) and conventional DCs (cDCs). pDCs are potent producers of type 1 interferons that drive immune responses in the context of viral infection. cDCs have been further subclassified into cDC1s and cDC2s (*2*). cDC1s induce activation of cytotoxic T cell responses through cytosolic antigen presentation or cross- presentation of exogenous antigens via class I major histocompatibility complex (MHCI) molecules. cDC2s are functionally more di- verse, equipped with a broad range of pattern recognition receptors, and specialized in the presentation of extracellular antigens via the MHCII pathway to induce $CD4^+$ T cell responses.

One challenge in studying DCs or harnessing their potential for clinical intervention to target the adaptive immune response against antigens of choice is that they are relatively rare immune cells, and the generation of DCs in large numbers remains a challenge. In the early 1990s, it was shown that the culture of mouse bone marrow (BM) cells or human peripheral blood mononuclear cells with granulocyte/macrophage- stimulating factor (GM-CSF), in combination with interleukin-4 (IL-4), generated cells with DC-like characteristics, including a typical dendritic morphology, membrane expression of CD11c, and MHCII molecules and the capacity to activate naïve allogeneic T cells (*3*, *4*). However, as our appreciation of DC heterogeneity advanced, it became apparent that DCs generated by using these culture conditions are, for the most part, descendants of monocytes, which are now termed monocyte-derived DCs (moDCs). moDCs show some in vivo similarities to migratory DCs and cDC2s but not to cDC1s (*5*). Indeed, cDCs are derived from a dedicated DC precursor in the BM and do not share a monocyte origin. Therefore, several questions arise from these results. How appropriate would it be to use moDCs as surrogates for cDCs? Are these moDCs efficient inducers of $CD8^+$ T cell responses in vivo, and can this lead to a longlasting immune memory? Have these moDCs acquired all the migratory, secretory, and antigen-presenting machinery that cDCs have? More recently, it has become feasible to generate cDC1-like cells by using culture systems that involve addition of FMS-like tyrosine kinase 3 ligand (FLT3L) and the Notch2 ligand d-like protein 1 (DLL1/DL1) (*6*, *7*). FLT3L is essential for cDC development in both human and mouse, and the absence of this cytokine leads to strong reduction in the number of cDCs but not monocytes. Although FLT3L/ DLL1-derived cDC1-like cells show stronger correlation to their in vivo counterparts, the generation of these cells for effective therapy would require large numbers of autologous BM stem cells or BM cDC precursors. Another possibility is the generation of DCs from induced-pluripotent stem cells (iPSCs). To date, attempts to generate DCs from iPSCs remain in their infancy.

Rosa *et al*. have used an alternative approach to reprogram fibroblasts directly into cDC-like cells (*1*). The authors investigated the ability of a set of candidate transcription factors to induce cDC-like cells directly from fibroblasts. They initially screened 18 transcription factors that are enriched in the DC lineage and tested their potential to induce reporter expression in embryonic fibroblasts isolated from *Clec9a-Cre X R26-stop-tdTomato* mice (*8*). Clec9a has been previously shown to be specifically expressed by cDC1 and, to a lesser extent, by cDC2 cells but not by other hematopoietic cells. This allowed the authors to use Clec9a reporter expression as a surrogate for the reprogramming of fibroblasts into cDCs. By combining smaller sets of transcription factors together, the authors identified three transcription factors, PU.1, IRF8, and BATF3, to be sufficient to directly reprogram mouse and human endothelial fibroblasts into cDC1-like cells that the authors have termed “induced” DCs (iDCs). Previous studies in mice have shown that both IRF8 and BATF3 are vital for the generation of cDC1 because the deletion of either gene leads to the almost complete absence of cDC1 cells in vivo (*9*, *10*).

Although the authors were able to reprogram mouse embryonic fibroblasts, human embryonic fibroblasts, and human dermal fibroblasts to iDCs, the authors chose to carry out much of the indepth analyses on mouse embryonic fibroblast–derived iDCs. Mouse iDCs exhibited a stellate morphology and high surface expression of MHCII and MHCI molecules and could be activated by TLR3 (polyinosinic:polycytidylic acid) or TLR4 (lipopolysaccharide) ligands. The authors also examined the ability of mouse iDCs to present antigen to induce a robust $CD4^+$ T cell response and, more importantly, to prime $CD8^+$ T cells responses by means of cross-presentation. As for human iDCs, the authors confirmed that these iDCs had cDC1-like characteristics, with phagocytic activity and MHCII (human lymphocyte antigen–DR) expression.

By carrying out single-cell RNA sequencing from iDCs on days 3, 7, and 9 of transdiffeentiation, the authors explored the relationships between PU.1, IRF8, and BATF3 in shaping the transcriptional program in iDCs. The authors found that transdifferentiation followed an asynchronous and stepwise pro- gram that terminates in stable commitment to the DC fate through the induction of endogenous *Irf8* and *Batf3* expression.

The author's transdifferentiation system to generate DCs is likely to be valuable in basic research and in the clinic. In terms of DC biology, this system should allow further in-depth exploration of underlying epi- genetic events during DC differentiation in both humans and mice. Because skin fibroblasts from patients are relatively easy to obtain, it is conceivable that this system could become important in the clinic to generate autologous, patient-specific iDCs for vaccines.

However, much remains to be learned about this PU.1-, IRF8-, and BATF3-dependent reprogramming. First and foremost, the stability of these cells in vivo and their antigen presentation abilities under pathological conditions remains to be examined. Whether injected iDCs can traffic to lymphoid organs and interact with immune and nonimmune cells in these organs remains to be seen. For clinical applications in which large numbers of autologous DCs are required, a direct comparison to FLT3L/DLL1-derived cDC1-like cells with respect to cell yield and costs is needed. Nonetheless, what is most exciting about the system developed by Rosa *et al*. is that it could be tweaked to generate subsets of DCs other than cDC1s. By generating DCs from fibroblasts, Rosa *et al*. have opened up the avenue of cellular reprogramming for the next generation of cellular vaccines.

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