Dedicated mTEC Progenitors Stay True, Even into Adulthood

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Knowledge about the cells giving rise to and maintaining the thymic structure remains limited. In this issue of *Immunity*, Sekai et al. (2014) identify a postnatal self-renewing unipotential progenitor population capable of generating thymic medullary cells and lay the foundation for research into thymic regeneration.

In vertebrates, the thymus is the key site of T cell lymphopoiesis. Its structure is composed, primarily, of thymic epithelial cells (TECs) arranged in a 3D reticular network (Petrie and Zúñiga-Pflücker, 2007). The thymus can be further subdivided into two main distinct functional regions: the cortex, where newly differentiated T cells are positively selected for the ability to respond to self-major histocompatibility complex (MHC) molecules, and the medulla, where positively selected T cells are interrogated against strong reactivity to self-antigens presented on the MHC. Such T cells undergo apoptosis, leaving only non-self-reactive T cells to mature and exit into the periphery. The importance of the thymus in adaptive cellular immunity has been known for over 50 years (Miller, 2014). However, it is only now that we are gaining a better understanding of the cell types that give rise to the structural components found within the thymus.

In 2002, the first studies examining the progenitor relationship of TEC lineages were published (Bennett et al., 2002; Gill et al., 2002). These studies, as well as others that followed, indicated the existence of a common TEC progenitor that can give rise to both cortical TECs (cTECs) and medullary TECs (mTECs). However, a definitive set of markers for such a progenitor has yet to be identified. Additionally, it is not known whether downstream of the common TEC progenitor there exists a lineage-restricted progenitor that gives rise to cTECs or mTECs (Gray et al., 2006). The ability of said progeny to undergo self-renewal throughout life in order to contribute to the dynamic thymic stromal environment is also unknown.

In this issue of *Immunity*, Sekai et al. (2014) tackle some of these unknowns by extending their earlier finding that a subset of embryonic TECs expressing high amounts of claudin-3 and claudin-4 (Cld3,4) represents an early progenitor of mTECs (van Ewijk et al., 1999). They now investigate whether such cells are present in the postnatal thymus, exhibit the stem-cell-like capability of self-renewal, and thereby contribute to the lifelong maintenance of the mTEC population within the adult. Their current findings demonstrate that when implanted in an athymic nude animal, Cld3,4^{hi} embryonic TECs will successfully generate a normal thymic medulla in which TECs express classical mTEC genes, such as *Aire* and *Krt5*. The medullary compartments were detectable in animals up to 18 months, supporting the notion that Cld3,4^{hi} TEC progenitors can indeed confer lifelong maintenance of the mTEC population. Additionally, the ectopic medulla of these animals was also functional and capable of directing the removal of self-reactive T cells.

Of note, although their results show promise for the use of Cld3,4^{hi} cells in the treatment of autoimmune disorders, such as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (in which *Aire* is defective, leading to mTEC dysfunction), it should be mentioned that the experimental approaches used by Sekai et al. involved the implantation of an ectopic reconstituted thymus into the kidney capsule, and this led to the removal of self-reactive T cells. This approach could be challenging in a clinical setting; a preferred treatment method would involve the restoration of a normal thymic medulla compartment within the original thymus. Therefore, it remains to be seen whether the injection of Cld3,4^{hi} cells directly into the thymus of autoimmune-prone animals with defective mTECs gives rise to a functional medulla to promote the deletion of self-reactive T cells from the repertoire and thus reestablish self-tolerance.

Because Cld3,4 are also expressed on mature TECs, Sekai et al. sought to find a second marker to prospectively isolate this progenitor pool in adult animals. They detected a small fraction of adult Cld3,4^{hi} TECs that also expressed stage-specific embryonic antigen 1 (SSEA-1), a marker of embryonic stem cells. Using in vitro assays, they showed that Cld3,4^{hi}SSEA-1^{+} TECs could form individual TEC colonies and that these colonies contained cells that when isolated could generate more TEC colonies. Of note, when implanted into athymic recipients, the Cld3,4^{hi}SSEA-1^{+} fraction gave rise to only the thymic medulla and not the cortex. The Cld3,4^{hi}SSEA-1^{-} cells could not establish either a cortex or a medullary compartment. These results were recapitulated with embryonic Cld3,4^{hi}SSEA-1^{+} cells as well.

These findings put forth compelling evidence that a unipotent mTEC stem cell, termed medullary thymic epithelial cell stem cell (mTECSC), is found within the Cld3,4^{hi}SSEA-1^{+} population of embryonic and adult mTECs and that these mTECSCs can maintain lifelong mTEC populations (Figure 1). Of interest, Sekai et al. also noted that the frequency and regenerative capacity of the mTECSCs decreased rapidly with age and were inversely correlated with exposure to developing thymocytes. Although Gray et al. demonstrated that the renewal capacity of the thymus diminishes rapidly with age (Gray et al., 2006), Sekai et al. add to this notion by putting forth evidence of the concept that exposure to

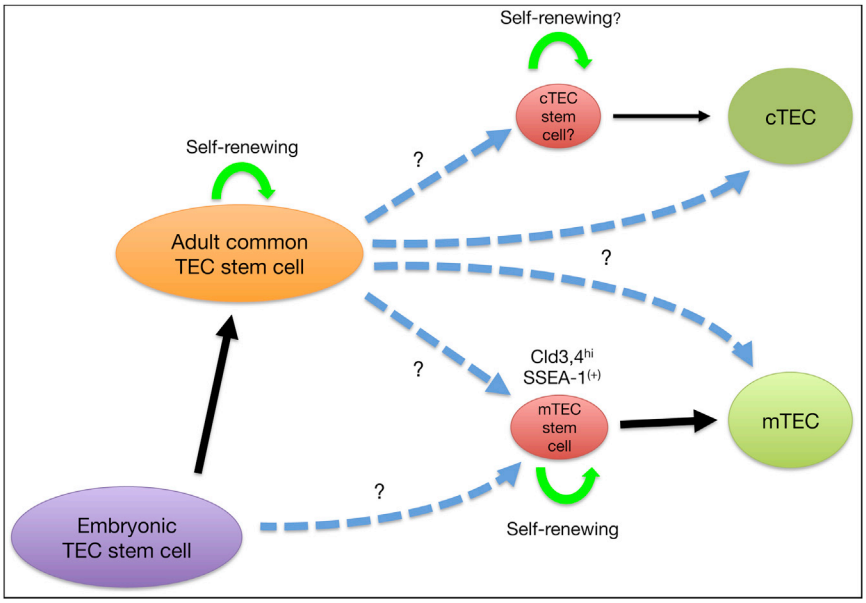

Figure 1. Schematic View of Progenitor-Progeny Relationships of Different TEC Subtypes
The adult thymus contains a self-renewing pool of stem cells, which can give rise to both cTECs and mTECs. Additionally, a pool of self-renewing Cld3,4^{hi}SSEA-1^{+} mTECSCs can give rise exclusively to mTECs. However, it is unknown whether these mTECSCs arise from the common adult TEC stem cell or whether they are embryonically derived. Additionally, the immediate progeny of the common TEC stem cell remains to be definitely identified.

developing thymocytes hastens this loss of regenerative capability. This view is counterintuitive to the longstanding view that stromal-lymphocyte interactions, known as cross-talk, are typically thought to have a positive effect on stromal function (van Ewijk et al., 1999).

Two other recent papers have demonstrated the existence of self-renewing TECSCs within adult thymi (Ucar et al., 2014; Wong et al., 2014). However, Ucar et al. and Wong et al. put forth evidence of bipotent TECSCs, capable of generating both cTECs and mTECs in vivo and in vitro. A common feature of TECSCs, and mTECSCs, is the expression of stem-cell-associated surface markers, such as Sca-1. In the context of the description of these adult TECSCs, it remains unclear whether mTECSCs share a common origin with TECSCs or whether they represent a distinct lineage of unipotent stem cells (perhaps embryonically derived), which would then support the adult pool of mTECs (Figure 1).

Of great interest, Ucar et al. took advantage of a cell-lineage-tracing approach to demonstrate that their adult TECSC population did not express the transcription factor FoxN1. It was only after differentiation that FoxN1 expression was observed. These results are intriguing given that FoxN1 has been shown to be expressed early in thymic ontogeny and is essential for proper thymic epithelial cell development (Gordon and Manley, 2011). The work by Ucar et al. suggests that FoxN1 expression might be unnecessary for the formation of the earliest TEC progenitors within the embryo and might be only expressed in order to achieve full differentiation of TECSCs into cTECs and mTECs. However, mTECSC colonies described by Sekai et al., which possessed long-term renewal capacity and could give rise to mTECs after adoptive transfer in vivo and retained the mTECSC phenotype, did indeed express FoxN1. The apparent discrepancy as to the requirement for FoxN1 expression by self-renewing TECSCs could be explained by the existence of two distinct stem cell pools or could be due to the embryonic versus adult source of the different cell types being investigated.

In summary, the work of Sekai et al. (2014) provides novel insights into the existence of self-renewing mTECSCs that can potentially be used as therapeutic targets for thymus regeneration in patients undergoing cytoablative therapies or in older individuals whose thymi have long since involuted. As usual, further experiments will be required for determining whether any of these applications are feasible in individuals and how best to enable the regenerative potential of mTECSCs.

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