THYMOCYTE DEVELOPMENT

Introducing … thymic tuft cells

Purging self-reactive thymocytes from the repertoire is regulated by a specialized stromal cell compartment in the thymic medulla, consisting mainly of medullary thymic epithelial cells (mTECs) that express tissue-restricted antigens (TRAs) under the control of AIRE. A growing appreciation of the heterogeneity of mTECs was the starting point for two studies that describe an mTEC subset with similarities to intestinal tuft cells.

Miller et al. used a lineage-tracing model in which cells that express or have expressed AIRE are inducibly labelled with RFP. Using bulk RNA sequencing (RNA-seq), they identified four mTEC subsets. MHC-II^{hi}RFP^{low} and MHC-II^{hi}RFP^{hi} subsets were closely related to each other and expressed the highest levels of *Aire* and TSA transcripts. The post-*Aire* expression MHC-II^{low}RFP^{+} population was shown to comprise two subsets: one subset expressed the cytoskeletal keratin KRT10; the other subset expressed genes that are associated with intestinal tuft cells. The thymic tuft cells were closely associated with KRT10^{+} structures in the medulla of mice, which are consistent with the cornified Hassall's corpuscles found in human thymus. Bornstein et al. carried out massively parallel single-cell RNA-seq (MARS-seq) of mTECs, resulting in their similar classification into four major groups. The mTEC I and mTEC II populations had features of immature *Aire*$^{-}$ and mature *Aire*$^{+}$ mTECs, respectively. The mTEC III population expressed KRT10, and the mTEC IV population expressed genes of the canonical taste transduction pathway (such as *Trpm5*) that are associated with tuft cells.

Bornstein et al. showed that the transcriptional profile of mTEC IV cells is more similar to that of intestinal tuft cells than any of the other TEC populations. Furthermore, they found that mTEC IV-specific enhancers are enriched for motifs that bind the tuft cell transcription factor POU2F3. Both groups showed that *Pou2f3*$^{-/-}$ mice have a specific loss of the tuft cell-like mTECs. Intestinal tuft cells are the sole source of the type 2 cytokine IL-25; Miller et al. confirmed tonic IL-25 production by the tuft cell-like mTECs, and Bornstein et al. showed that mTEC IV cells express even higher levels of IL-25 than intestinal tuft cells.

Looking at the development of these thymic tuft cells and their relationship to the other mTEC subsets, the results of the in vivo fate mapping used by Bornstein et al. suggest that mTEC IV cells appear only after birth and are derived from mTEC II and/or III cells or a common ancestor, which is consistent with the lineage-tracing findings of Miller et al. Although Miller et al. showed that thymic tuft cells pass through an *Aire*-expressing stage, both groups found that thymic tuft cells can develop in an *Aire*-independent pathway.

Thymic tuft cells were also shown to express a broad array of type II taste receptor (TAS2R) family members by Miller et al., with the nonrandom expression of *Tas2r* gene pairs by individual cells suggesting that different tuft cell subsets may have different chemosensitivities. Whereas Bornstein et al. showed that thymic tuft cells lack stochastic expression of AIRE-dependent TRAs, Miller et al. described a unique ability of these cells to express MHC class II molecules and associated antigen presentation genes (compared with intestinal tuft cells, which do not express MHC class II).

Given the unique gene expression profile of thymic tuft cells that both groups described, they set out to examine the function of these cells in the thymus. Both groups looked at the function of thymic tuft cell-derived IL-25 in driving type 2 responses. Bornstein et al. approached this by applying unbiased single-cell RNA-seq of all IL-25R^{+} thymic immune cells; paradoxically, they showed that the loss of thymic tuft cells in *Pou2f3*$^{-/-}$ mice was accompanied by an increase in the number of IL-25R^{+} thymus-resident group 2 innate lymphoid cells (ILC2s). By contrast, Miller et al. described a marked decrease in the number of type 2 invariant natural killer T (NKT2) cells in *Pou2f3*$^{-/-}$ mice, as well as decreased IL-4 production by these cells and a decreased number of IL-4-dependent EOMES^{+}CD8^{+} thymocytes. *Trpm5*$^{-/-}$ mice had a similar phenotype, which showed that the taste transduction pathway is required for the function of thymic tuft cells. Finally, Miller et al. also showed that thymic tuft cells could impose tolerance to the tuft cell-restricted antigen IL-25 in thymic grafting experiments.

> “the taste transduction pathway is required for the function of thymic tuft cells”

In summary, both groups have described a small population of mTECs with tuft cell properties. Together, the results suggest that these cells occupy a unique microanatomical niche, with a unique ability to sense micro-environmental cues that is required for their function. They may have a role in inducing tolerance to tuft cell-restricted antigens as well as in regulating type 2 responses within the thymus, although the details will require further study.

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ORIGINAL ARTICLES Miller, C. N. et al. Thymic tuft cells promote an IL-4-enriched medulla and shape thymocyte development. *Nature* https://doi.org/10.1038/s41586-018-0345-2 (2018) | Bornstein, C. et al. Single-cell mapping of the thymic stroma identifies IL-25-producing tuft epithelial cells. *Nature* https://doi.org/10.1038/s41586-018-0346-1 (2018)