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INSIGHTS

Thymic mimicry: The art of imitation

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Display of tissue self-antigens within the thymus is critical for the regulation of self-reactive T cells. In this issue of *JEM*, Michelson et al. (2023. *J. Exp. Med.* https://doi.org/10.1084/jem.20230461) continue to advance our understanding of self-antigen representation by medullary thymic epithelial cells, identifying a new role for Hnf4γ in the regulation of thymic mimetic cells as well as their peripheral counterparts.

Silencing of self-reactive T cells is essential to prevent the development of potentially lethal autoimmunity. Within the thymus, developing thymocytes enter a rigorous selection process that ensures the removal of T cells with high affinity for self-antigen, or their diversion to immunosuppressive regulatory T (Treg) cells. A conundrum that puzzled immunologists for decades was how T cells could be screened for reactivity against the full repertoire of self-proteins, many of which are expressed in a tissue-restricted manner. An elegant solution was provided by the discovery that medullary thymic epithelial cells (mTECs) had a unique ability to ectopically express thousands of tissue-restricted antigens (TRAs; Derbinski et al., 2001). In a landmark study, Mathis and colleagues identified the Aire protein as the key nuclear factor driving ectopic TRA expression in mTECs (Anderson et al., 2002). The clinical significance of this finding cannot be overstated, as mice and humans deficient in Aire expression develop widespread autoimmunity (Aaltonen et al., 1997; Nagamine et al., 1997; Anderson et al., 2002). This discovery shaped our understanding of central tolerance and spurred decades of research into the mechanism of Aire regulation of TRAs. Initial single-cell analyses suggested that TRA induction is stochastic with ∼1–5% of mTECs expressing a given TRA with no clear tissue pattern among TRAs expressed by an individual mTEC (Derbinski et al., 2008; Brennecke et al., 2015; Meredith et al., 2015). However, more recent single-cell genomic analyses added a new dimension to our understanding of thymic self-antigen expression, uncovering previously unappreciated heterogeneity among mouse and human mTECs, most notably an array of rare mTEC subsets that resemble peripheral cell types (Bornstein et al., 2018; Baran-Gale et al., 2020; Park et al., 2020; Bautista et al., 2021; Michelson et al., 2022), termed mimetic cells (Michelson et al., 2022). In contrast to Aire$^+$ mTECs, which express peripheral antigens in a seemingly random manner, mimetic cells are defined by expression of lineage-specific transcription factors (TFs) that promote expression of corresponding peripheral cell transcriptional programs and thus TRAs in a coordinated manner (Michelson et al., 2022). Intriguingly, the majority of mimetic cell sub-types develop from Aire-expressing mTECs, and in some cases are dependent on Aire (Wells et al., 2020; Abramson et al., 2022 *Preprint*; Michelson et al., 2022). However, the exact mechanism by which Aire$^+$ mTECs gain pluripotency and the signals that drive expression of lineage-determining TFs are not known.

The discovery of thymic mimetic cells opens a new chapter in the investigation of mTEC biology and raises a number of key questions: What is the ontogeny of mimetic cells? What are the extrinsic and intrinsic regulators that promote distinct mimetic cell fates? And, perhaps most importantly, what is the relative contribution of Aire$^+$ mTECs versus mimetic cells to self-tolerance? Building on their recent discovery and characterization of thymic mimetic cells, Michelson and colleagues set out to address the molecular mechanisms that drive mimetic cell differentiation and function, focusing on entero-hepato mTECs that share transcriptional features with gut and liver epithelial cells (Michelson et al., 2023). The authors defined a shared transcriptional program between entero-hepato mTECs and their peripheral counterparts that included Hnf4 family members. Within the periphery, enterocytes are redundantly dependent on Hnf4α and Hnf4γ for their differentiation (Chen et al., 2019), whereas hepatocytes require Hnf4α (Parviz et al., 2003), leading the authors to focus on the role of these lineage-determining TFs in entero-hepato mTEC differentiation. Using a series of single and combinatorial, conditional, or global Hnf4α and Hnf4γ

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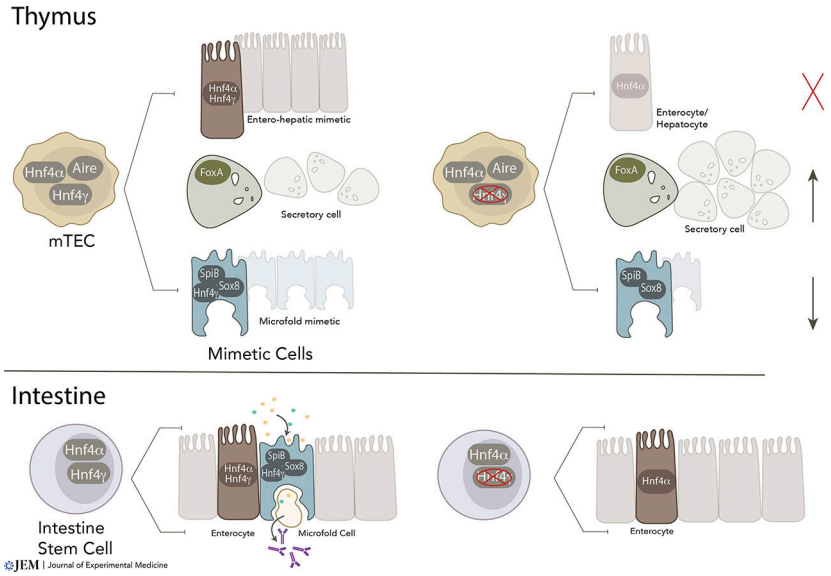

Hnf4γ regulates the differentiation of mTECs. Deletion of Hnf4γ leads to loss of entero-hepato mTECs and a partial reduction in thymic M cells. Within the intestine, gut M cells were also found to be dependent on Hnf4γ, revealing symmetry between thymic mimetic cells and their peripheral counterparts.

knock-out mice, the authors show that entero-hepato mTEC differentiation is critically dependent on Hnf4γ but not Hnf4α. In addition, ablation of Hnf4γ led to a partial loss of mTECs that mimic gut microfold (M) cells (see figure), prompting the authors to examine the role of Hnf4γ in their peripheral counterparts. Analysis of the intestine revealed a previously unappreciated role for Hnf4γ in gut M cell differentiation with loss of M cells and associated defects in Peyer's patch IgA$^+$ B cells. Thus, a striking feature of this study was the fidelity with which thymic mimetic gene expression programs and their transcriptional regulators mirrored their intestinal counterparts, demonstrating the power of mimetic cell investigation to reveal novel biology in peripheral tissues.

Consistent with the central role of Hnf4γ in entero-hepato mTEC differentiation, analysis of Hnf4γ-bound chromatin revealed extensive binding in mTECs including a large number of sites that were exclusively bound in mTECs relative to peripheral enterocytes. A key question that arises is what promotes the expression and binding of Hnf4γ in mTECs. Aire, with its unusual ability to promote promiscuous gene expression, is a prime candidate; however, despite the high degree of overlap identified between Aire and Hnf4γ binding sites, prior work by Michelson and colleagues demonstrated that entero-hepato mTEC differentiation was not impacted by loss of Aire. Moreover, in this study, Hnf4γ expression was not affected by loss of Aire. Comparison of chromatin features at Hnf4γ-bound peaks in entero-hepato mimetic cells provided a potential clue to the upstream regulators of Hnfγ, revealing enrichment of NF-κB motifs. Given the dual roles of receptor activator of the NF-κB ligand (RANKL)/RANK/NF-κB signaling in Aire$^+$ mTEC differentiation (Rossi et al., 2007) and intestinal M cells (Knoop et al., 2009), the authors speculate that RANKL may play a role in Hnf4γ mediated mTEC differentiation. Further elaboration of this signaling pathway will be an exciting avenue for future research.

What is the purpose of having two distinct pathways for self-representation in the thymus, and do they instruct qualitatively distinct aspects of immune tolerance? While both pathways lead to negative selection (Michelson et al., 2022), investigations addressing the relative contribution of stochastic vs. coordinated TRA expression to peripheral T cell tolerance are in their infancy. A notable feature of mimetic cells is their relative abundance, with increased representation of cells that mimic those where immune tolerance is paramount, notably the skin, gut, and lungs. Perhaps an evolutionary advantage arises in having a dual system of coverage for antigens expressed by tissues most susceptible to tolerance breakdown and autoimmunity. Alternatively, Aire$^+$ mTECs and mimetic cells may serve distinct functions, either through division of TRA expression, or through distinct roles in instructing clonal deletion vs. Treg cell differentiation. In this study, the authors show that loss of entero-hepato mimetic cells resulted in lymphocytic hepatic infiltration at homeostasis, despite lack of liver-specific autoantibodies, as well as increased susceptibility to chemical-induced intestinal epithelial injury. These findings suggest a non-redundant role for entero-hepato mimetics cells in peripheral tolerance. However, the serendipitous finding of Hnf4γ-dependent M cell differentiation, as well as the recently described role for Hnf4γ in intestinal epithelial lymphocyte differentiation (Song et al., 2023), preclude definitive conclusions regarding autoimmune inflammation in Hnf4γ-deficient mice, given the established role of these cell types in intestinal homeostasis. Further studies are needed to tease apart individual contributions of each mimetic cell type, including the shared or distinct TRAs expressed by Aire$^+$ mTECs and mimetic cells.

Since the discovery of Aire over 20 yr ago, studies of central tolerance have focused on Aire and its role in mTEC mediated self-tolerance. These studies have informed our framework for thymic selection and T cell tolerance. The discovery of mimetic cells emphasizes once again the mysterious nature of mTECs, adding an exciting and intriguing dimension to mTEC biology that must be factored into our models for thymic tolerance. The present findings from this study provide a detailed transcriptional and epigenetic characterization of entero-hepato mimetic cells underscoring the importance of lineage determining TFs in both thymic mimetic and intestinal epithelial cell differentiation. Yet the cues that drive expression of these TFs and the mechanisms underlying the permissive nature of Aire$^+$ mTECs to reprogramming remain enigmatic. At present, the identity of the mimetic progenitor, or perhaps multiple mTEC progenitors that give rise to distinct mimetic cell types, is not known. Thus, while mTECs presently retain their air of mystery, future studies of both Aire-mediated gene expression and mimetic cell differentiation may unveil new mechanisms of transcriptional regulation and enhance our understanding of tissue development and autoimmunity.

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