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Thymic mimetic cells: tolerogenic masqueraders

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Abstract

Medullary thymic epithelial cells (mTECs) clonally delete or divert autoreactive T cells by ectopically expressing a diverse array of peripheral-tissue antigens (PTAs) within the thymus. Although thymic stromal cells with histological features of extra-thymic cell types, like myocytes or neurons, have been observed by light microscopy since the mid-1800s, most modern work on PTA expression has focused on the transcription factor Aire. Here, we highlight recent work that has refocused attention on such “misplaced” thymic cells, referred to collectively as thymic mimetic cells. We review the molecular underpinnings of mimetic cells and their roles in establishing T-cell tolerance, and we propose that mimetic cells play important roles in autoimmunity. Finally, we suggest future directions for this emerging area.

Peripheral-tissue antigen expression in thymic epithelial cells

All stages of pre-immune T-cell maturation in vertebrates, including T-cell lineage commitment, T-cell receptor (TCR) formation, $CD4^+$ vs $CD8^+$ lineage choice, **positive selection, negative selection**, and **agonist selection** (see Glossary), occur within the thymus, with thymic epithelial cells (TECs) playing key instructive roles throughout (reviewed in [1]). In the cortex, **cortical TECs** (cTECs) provide signals for lineage choice and major histocompatibility complex (MHC) restriction, while in the medulla, **medullary TECs** (mTECs) express **peripheral-tissue antigens** (PTAs) to preview the peripheral self to maturing thymocytes. The latter process allows for identification of T cells bearing autoreactive TCRs and their clonal deletion or diversion into the **regulatory T cell** (Treg) lineage, thereby enforcing **central tolerance**.

Since the original observation of PTA expression by mTECs [2-4], the molecular basis of this unusual phenomenon has received considerable attention. Some early models postulated a relationship to histologically distinct epithelial cells that had been observed in the medulla since the mid-1800s [5], long before the role of the thymus or even basic principles of adaptive immunity were understood. These “misplaced” cells included skin-like Hassall’s corpuscles, lung-like ciliated cysts, and muscle-like myoid cells, to name a few (**see** Box 1)

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[6-8]. However, the transcription factor (TF) **Aire** was soon implicated as a major regulator of PTA expression, with population-level transcriptomic analyses of mTECs from *Aire*$^{-/-}$ mice showing decreased expression of most PTAs [9]. Consistent with a major role for Aire in central tolerance, mice and humans with mutations in the *Aire/AIRE* gene developed autoantibodies against Aire-dependent antigens and multiorgan lymphocytic infiltration [3,9-13]. These results shifted the focus of studies on PTA expression to Aire-dependent mechanisms, and interest in the early histologic observations waned.

Here, we briefly review early studies on PTA expression and Aire before highlighting recent work that has refocused attention on “misplaced” thymic stromal cells, collectively termed mimetic cells. We discuss molecular, cellular, and immunological aspects of mimetic cells, and argue that mimetic cells likely play important roles in the pathogenesis of autoimmunity. Finally, we suggest future directions for this emerging area.

The Aire model of PTA induction

While compelling in many respects, the Aire model of PTA induction left several questions unanswered. First, Aire has little-to-no sequence-specific binding activity [14]; how, then, could it select genes to induce, especially given the diverse and disparate nature of its targeted genes? Second, the expression of many PTAs is diminished, but not extinguished, in *Aire*$^{-/-}$ mTECs [15,16]; might Aire act as an amplifier of PTA expression, rather than its primary inducer? Finally, individual PTAs, far from being expressed in all mTECs, are instead expressed in a variegated fashion, with any given PTA typically expressed in only 1-5% of mTECs [4]; why would Aire, which is uniformly expressed in a major fraction of mTECs, induce PTAs so heterogeneously?

Especially given this last point, single-cell transcriptomics were intuitively appealing to study the landscape and mechanism of PTA expression. Early work in this area used single-cell reverse transcription and polymerase chain reaction (RT-PCR) and plate-based single-cell RNA sequencing (scRNA-seq) to examine PTA coexpression patterns in murine and human mTECs [16-20]. In general, though, these studies were limited by throughput constraints and, although some non-randomness in PTA expression was observed, few coherent patterns emerged, leading to the consensus that PTA expression in mTECs was “quasi-random” and proceeded with “ordered stochasticity.” Contemporaneously, several molecular studies characterized Aire’s influence on gene regulatory mechanisms, such as RNA polymerase II pausing, epigenetic modifications, RNA splicing, super-enhancer activity, and chromatin looping [16,21-26]. From this work, the idea emerged that Aire could repurpose general transcriptional mechanisms to induce tissue-specific genes.

Defining mimetic cells

In parallel with these Aire studies, a few investigators continued to study the “misplaced” cells of the thymic medulla. Several microscopic and RT-PCR-based studies showed that the murine thymus contains clusters of cells resembling lung, thyroid, and parathyroid epithelia [27-30], raising the possibility that mTECs might appropriate the developmental programs of peripheral tissues for tolerance. However, no evidence of a functional role

for these cells in tolerance was provided, and a subsequent study found that TEC-specific conditional deletion (*Foxn1*^{cre}) of *Pdx1*, a pancreatic lineage-defining TF, did not affect mTEC expression of pancreatic genes [31]. Meanwhile, contemporaneous work on Aire continued to confirm its importance in PTA induction, eventually leading to a widespread consensus that Aire, rather than appropriated developmental mechanisms, was the major driver of PTA induction.

The tide shifted with the advent of higher-throughput scRNA-seq, which uncovered rare transcriptomic equivalents of histologically defined mTECs, including ciliated, myoid, and tuft mTECs [32-37]. These findings once again foregrounded the possibility of compartmentalized, non-random PTA expression. Single-cell characterizations of tuft mTECs were especially prescient, as the combined observations of two studies went beyond phenomenology to show that tuft mTECs relied on a tuft-cell lineage-defining TF, Pou2f3, for their accumulation, could mediate tolerance to a tuft-cell-restricted antigen, and had influences on thymic cell subsets beyond conventional αβ T cells [32,33].

Recently, based on the single-cell assay for transposase-accessible chromatin and sequencing (scATAC-seq), our own study of chromatin accessibility in individual mTECs systematized these individual cell-type observations to describe a whole constellation of peripheral-cell-mimicking mTECs with principled differentiation and PTA expression [38]. We found that the accessible chromatin of mTEC subsets was enriched for the signatures of lineage-defining TFs from peripheral cell types, and we hypothesized that these TFs might be co-opted by mTECs to drive expression of lineage-specific gene programs within the thymus. Indeed, targeted scRNA-seq of mTECs found numerous mTEC subtypes expressing paired lineage-defining TFs and lineage-specific programs, such as mTECs resembling *Grhl*$^{+}$ keratinocytes, *Foxa*$^{+}$ neuroendocrine cells, *Foxj1*$^{+}$ ciliated cells, *Hnf4*$^{+}$ enterocytes/hepatocytes, *Spib*$^{+}$ *Sox8*$^{+}$ microfold (M) cells, *Foxi*$^{+}$ ionocytes, *Myog*$^{+}$ skeletal muscle, *Spdef*$^{+}$ goblet cells, and *Ptf1a*$^{+}$ pancreatic secretory cells[38]. Many subtypes corresponded to mTECs that had been phenomenologically described by histology or scRNA-seq [6-8,34-37], and, as we sequenced more cells, more subtypes continued to emerge. Of note, another preliminary study, recently reported in preprint, found a similar diversity of mTECs using paired scATAC-seq and scRNA-seq [39]. Collectively, we use the term “mimetic cells” to refer to these mTECs with the lineage-defining TFs, chromatin landscapes, and gene expression programs of peripheral cell types (Box 1).

Principles of mimetic cells

Synthesis of our study and others has revealed some general principles of mimetic cell biology. Most fundamentally, mimetic cells have biologically logical PTA expression patterns controlled by lineage-defining TFs. Lineage-defining TFs correlate with ectopic expression of lineage-restricted programs within mimetic cells [38,39]; lineage-defining TFs bind specifically to the accessible chromatin of their corresponding mimetic cells [38]; and TEC-intrinsic loss of lineage-defining TFs results in loss of the corresponding mimetic cells, as shown by thymic graft experiments with *Spib*$^{-/-}$ or *Sox8*$^{-/-}$ murine thymi lacking microfold TFs [38] and by TEC-specific conditional deletion (*Foxn1*^{cre}) of the neuroendocrine TFs *Insm1* or *Ascl1* [39].

As concerns the provenance of mimetic cells, lineage tracing with *Aire*^{CreERT2} and *Csnb*^{Cre} mice has shown that most mimetic cells (excepting muscle mTECs) differentiate from Aire-expressing progenitors [33,38-40]. Importantly, however, differentiation of mimetic cells downstream of Aire does not imply that they require Aire for their differentiation. On the contrary, many mimetic cell types are numerically reduced, but not absent, in *Aire*$^{-/-}$ mice, giving a satisfying explanation for why, in population-level transcriptomic studies of mTECs, loss of Aire diminishes, but does not extinguish, the expression of many PTAs [15,33,38,39]. Aire does not appear to directly transactivate mimetic-cell PTAs, as gene expression within mimetic cells is largely unperturbed in *Aire*$^{-/-}$ mice [38,39], and Aire is not strictly required for binding of lineage-defining TFs to mimetic-cell chromatin, though it does enhance the binding of some TFs, such as Grhl1 and Pou2f3 [38].

To what extent do mimetic cells transdifferentiate into their peripheral counterparts? Many mimetic cells take on morphological features of their counterparts, such as polarized cilia in ciliated mTECs, lymphocyte pockets in microfold mTECs, and apical tufting in tuft mTECs [27,32,33,38]. Nonetheless, RNA-seq of mimetic cells and their peripheral counterparts show that mimetic cells retain the mTEC gene program as their core identity, with their cell-type-specific programs layered on as a minor (albeit substantial) fraction of their transcriptomes [33,38]. Spatially, immunofluorescent microscopy of murine and human thymi has shown that some mimetic cells (e.g., microfold and muscle mTECs) are scattered evenly through the thymic medulla, whereas others (e.g., ciliated, keratinocyte, and tuft mTECs) form coordinated microstructures such as respiratory cysts and Hassall's corpuscles [33,34,38]. A random distribution of mimetic cells would seem to be most efficient for negative selection by maximizing the likelihood of a thymocyte encountering its cognate antigen, but mimetic-cell microstructures may also serve as unique niches for specific thymic processes: for example, histologic analysis and *in vitro* co-culture experiments have suggested that human Hassall's corpuscles facilitate Treg induction by dendritic cells (DCs) [41].

Finally, mimetic cells have been shown in specific instances to be necessary and sufficient for antigen-specific T-cell tolerance. Transplantation of *Pou2f3*$^{-/-}$ thymi into nude mice led to the development of autoantibodies against the tuft-specific antigen, IL-25, and conditional deletion of *Insm1* and *Ascl1* in mTECs led to the development of autoantibodies against the thyroid and the enteroendocrine-rich gastric fundus [39], demonstrating the necessity of mimetic cells for tolerance [33]. Conversely, induced expression of a model self-antigen, yellow fluorescent protein (YFP), in ciliated and muscle mTECs using lineage-specific Cre drivers (*Foxj1*^{Cre}, *Ckm*^{cre}) diminished the number of YFP-reactive T cells in the periphery, indicating that self-antigen expression in mimetic cells suffices for tolerance [38]. Extrapolating from these data, we argue that mimetic cells are likely to be the primary drivers of thymic tolerance for PTAs whose expression is restricted to mimetic cells.

An integrated model of PTA expression

The newly recognized role of mimetic cells should not be taken to negate the well-established importance of Aire in central tolerance. Rather, the two mechanisms require integration to provide a more comprehensive view of thymic selection. Several key insights

into Aire function, gleaned from our recent study and elsewhere, inform on its role in our integrated model. First, not surprisingly, Aire exerts its direct effects where it is expressed, within Aire-stage mTECs, which have a chromatin and transcriptional state distinct from that of mimetic cells [38]. At the chromatin level, Aire enhances accessibility at Aire-binding sites and Aire-induced genes, without altering accessibility at Aire-neutral genes or at active or repressive histone marks more broadly [25,38]. Notably, these results are not in agreement with the recently proposed notion, based on bulk ATAC-seq data, that Aire acts primarily as a repressor, an interpretation that we suggest was confounded by bulk amalgamation of primary and secondary effects of Aire on mTEC chromatin [42]. At a transcriptional level, Aire induces diverse PTAs, unique to Aire-stage mTECs, and with a predilection for genes encoding inflammatory/antimicrobial peptides such as *S100a8, S100a9*, and *Defb19*, as well as neuropeptides such as *Ins2, Gip*, and *Ppy* [38,39].

Altogether, we envision a model (Figure 1, Key Figure) in which transit-amplifying TECs differentiate into Aire-stage mTECs, which express high amounts of MHC class II molecules and a select set of PTAs that are directly induced by Aire through repurposing of general transcriptional mechanisms and cooperation with pre-expressed mTEC TFs. At some subsequent point, diverse lineage-defining TFs are induced by Aire, spatial cues, and/or other signals. Once expressed, lineage-defining TFs drive the differentiation of diverse mimetic cell types, producing and maintaining their chromatin states and transcriptional programs, including mimetic-cell-specific PTAs. Maturing autoreactive thymocytes can undergo negative or agonist selection against the full range of PTAs expressed by Aire-stage mTECs and mimetic cells, which collectively encompass what were previously thought of as “Aire-induced” PTAs (Figure 2). Finally, defects in the thymic action of Aire or specific TFs lead to specific PTA deficiencies, giving rise to specific syndromic manifestations of autoimmunity.

Future perspectives

Many new questions have arisen (see Outstanding Questions). Some key areas of future work include comprehensively cataloging and characterizing mimetic cells, unraveling the molecular relationship between lineage-defining TFs and Aire, elucidating the impacts of mimetic cells on other cell types, and testing the role of mimetic cells in mouse and human autoimmunity.

Comprehensively characterizing mimetic cells

Most fundamentally, we need a comprehensive understanding of every mimetic cell type: their progenitors, relative abundances, associated PTAs, driving TFs, proliferative potential, capacity to transdifferentiate into other mimetic cell types, effects on T cells and other cells, and immunological consequences when deleted or defective. This last point is of particular interest as the consequences of mimetic cell dysfunction may inform a new understanding of autoimmune disease. For many of these experiments, new surface markers and genetic tools will need to be developed to permit facile analysis of the diverse mimetic cell types.

Molecular regulation of PTA expression

From a molecular perspective, we await a fuller picture of how Aire and lineage-defining TFs collaborate, or not, on mTEC chromatin to present a full diversity of PTAs to maturing thymocytes. Biochemically, Aire has chromatin-binding, rather than DNA-binding properties, suggesting that it may function more as a coactivator than a traditional TF [14,43-45]. Future experiments should explore whether Aire truly "chooses" its genes, or whether it is steered in all cases by sequence-specific TFs and/or site-specific signals. The chromatin of mTECs also seems uniquely permissive to the action of Aire and lineage-defining TFs, a principle established by early experiments showing stronger effects of Aire on gene expression in mTECs compared to other cell types and now underscored by observations of mTEC transdifferentiation into mimetic cells [38,46]. Comparative studies of mTECs, stem cells, and peripheral epithelia may reveal *cis*- and *trans*-regulatory features that permit the induction of a diverse repertoire of PTAs in mTECs while restraining the wholesale conversion of mTECs into *bona fide* tissues or teratomas.

Cellular functions of mimetic cells

At the cellular level, Aire-stage mTECs and mimetic cells represent clearly distinct cell states, raising the question of what the advantage of two mechanisms of PTA expression might be. One idea is that Aire-stage mTECs express a broad swath of PTAs but may not efficiently express inflammatory or other environmentally induced pathways for tolerization, whereas mimetic cells may coordinately induce pathways associated with their respective cell types, such as type 2 alarmins made by tuft cells, and Peyer's patch chemokines made by microfold cells [38]. Additionally, and not mutually exclusively, antigens derived from Aire-stage mTECs and mimetic cells may differ in their modes of presentation, thereby diversifying the peptide pool available for tolerance (i.e, differential peptide processing, differential use of MHC molecules, direct vs indirect presentation). Such a mechanism might be akin to the function of the thymoproteasome in cTECs [47]. These hypothetical possibilities should be assessed experimentally for their importance in central tolerance induction.

Beyond providing PTAs to conventional αβ T cells, mimetic cells may also play other roles in thymus biology. Hints of such roles have already emerged: *Pou2f3*$^{-/-}$ thymi lacking tuft mTECs show increased numbers of innate lymphoid group cells (ILC) but decreased NKT cells [32,33], and *Spib*$^{-/-}$ and *Sox8*$^{-/-}$ thymi lacking microfold mTECs show increased numbers of thymic B cells, but impaired generation of thymic IgA^{+} plasma cells [38,39]. Many mimetic cell types express transcripts encoding molecules that may influence their surrounding milieu, such as *Il10* and *Il25* by tuft mTECs and *Ccl6*, *Ccl20*, and *Tnfrsf11b* by microfold mTECs [32,33,38,39]. Alongside characterizations of conventional αβ T-cell repertoires, then, mice with defects in mimetic cells should also be evaluated for any impacts on unconventional T cells and other thymic cell subsets.

Mimetic cells and autoimmunity

While nearly all work to date on mimetic cells has been in mice, the coordinated provision of PTAs by mimetic cells has important implications for our understanding of autoimmune syndromes in humans. Much basic science work has shown that defects in central tolerance can causally cascade into autoimmunity, such as classic studies showing that variation in the number of tandem repeats at the insulin promoter can diminish thymic insulin expression and central tolerance to insulin, resulting in anti-insulin T cell and autoantibody responses and ultimately type 1 diabetes [48-50]. Clinically, however, most polygenic autoimmune diseases are still treated as phenomenological syndromes rather than as rooted in causal molecular defects, at least in part because few theoretical frameworks to understand autoimmune risk have emerged beyond polymorphism at human leukocyte antigen (HLA) loci.

We propose that mimetic cells may provide exactly such a framework: defects in the thymic action of lineage-defining TFs would lead to loss of tolerance to biologically coherent sets of antigens, resulting in biologically coherent patterns of autoimmunity. Indeed, lineage-defining TFs from mimetic cells have already been identified as risk loci in many human autoimmune diseases, including *HNF4A* in inflammatory bowel disease, *SPIB* in primary biliary cirrhosis, and *SOX8* in multiple sclerosis [51-53]. Moreover, mounting evidence suggests that HLA-linked autoimmune risk operates in large part within the thymus, during the establishment of central tolerance, providing support in favor of a “central hypothesis” for autoimmunity [54-57]. Human mimetic cells, some subsets of which have already been observed [36-38], should be evaluated for their adherence to the principles established in mouse and to test the hypothesis that mimetic cells are compromised in certain autoimmune syndromes.

Concluding remarks

The discovery of a constellation of mimetic cells in the thymus has substantially widened our view of mTEC biology and T-cell tolerance; nonetheless, we still have much to learn about these “tolerogenic masqueraders” (see Outstanding Questions). We now understand that Aire and lineage-defining TFs work together to induce PTAs in a principled and biologically logical fashion, as opposed to quasi-random induction by Aire alone. Moreover, proof-of-principle experiments have demonstrated that mimetic cells are important for self-tolerization of maturing thymocytes. Comprehensive characterizations of mimetic cells and their relationships to other thymic cell types can help reveal the influence of mimetic cells on the immune system and their importance in controlling autoimmunity. Studies of this remarkable phenomenon may also yield new insights into transcriptional regulation and developmental biology more generally.

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GLOSSARY

Agonist selection
Process by which some T-cell lineages, like Tregs and CD8αα$^+$ T cells, are induced via a positive interaction between their TCRs and cognate peptide:MHC complexes.

Aire
Primarily thymus-expressed TF that upregulates the expression of several thousand PTAs within mTECs. Mutations in Aire causally underlie the human autoimmune syndrome APECED (autoimmune polyendocrinopathy, candidiasis, and ectodermal dystrophy), also known as APS-1 (autoimmune polyglandular syndrome, type 1).

Central tolerance
Process of eliminating lymphocytes that react against self-antigens; for T cells, this occurs through negative selection in the thymus. Central tolerance is augmented by peripheral tolerance, which restrains autoreactive lymphocytes that escape central tolerization.

Cortical thymic epithelial cell (cTEC)
located within the thymic cortex; responsible for the induction of T-cell lineage commitment and positive selection into the αβ T cell lineage.

Lineage-defining transcription factor
Protein that controls gene expression required for the differentiation, diversification, maintenance, and/or survival of a specific cellular lineage.

Mimetic cell
mTEC that mirrors the lineage-defining TFs, chromatin-accessibility landscape, and gene-expression program of an extra-thymic cell type.

Medullary thymic epithelial cell (mTEC)
located within the thymic medulla; responsible for the induction of PTAs, some negative selection of maturing T cells, and agonist selection of Tregs and unconventional T cells.

Negative selection
Process by which T cells bearing autoreactive TCRs recognize their antigens on thymic antigen-presenting cells, including mTECs, and are clonally deleted from the T-cell repertoire.

Peripheral-tissue antigen (PTA)
Protein whose expression is normally confined to extra-thymic tissues (e.g., insulin, myelin basic protein, and mucin). mTECs express thousands of PTAs.

Positive selection
Process by which newly generated T cells are tested for the ability to interact with self-MHC molecules. T cells that successfully interact with self-MHC molecules continue to mature, while T cells lacking interaction undergo death by neglect.

Regulatory T cell (Treg)

Foxp3^{+}CD4^{+} T cell that can suppress immune responses and regulate tissue homeostasis.

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BOX 1:

Mimetic cell types.

Basal (skin/lung) mTEC:

mimetic cells (MC) resembling basal cells of the skin and lung that putatively give rise, as in the periphery, to more terminally differentiated MC, such as keratinocyte mTECs and secretory/ionocyte mTECs [38].

Enterocyte/hepatocyte mTEC:

Hnf4a^{+} *Hnf4g*$^{+}$ MC expressing transcripts associated with gut enterocytes and liver hepatocytes, such as *Vil1, Aldob* and *Apoa4* [38]. These cells putatively give rise to microfold mTECs [38].

Ciliated mTEC:

Foxj1^{+} *Rfx2*$^{+}$ *Trp73*$^{+}$ MC exhibiting polarized cilia, line respiratory cysts in the thymus, and expressing transcripts associated with ciliated cells, such as *Dynlrb2, Pifo* and *Tubb4b* [7,28,34,38]. Expression of a model antigen in ciliated mTECs is sufficient to induce cognate T-cell tolerance [38].

Ionocyte mTEC:

Foxi1^{+} *Foxi2*$^{+}$ MC expressing transcripts (*Cftr, Slc12a2, Atp6v1b1*) associated with ionocytes, and ion-channel-rich cells found in the kidney and lung epithelium [37,38].

Keratinocyte mTEC:

Grhl1^{+} MC morphologically resembling skin keratinocytes, producing microscopically detectable Krt10^{+} cornified bodies that in humans have long been known as Hassall's corpuscles [6,33,38].

Microfold mTEC:

Spib^{+} *Sox8*$^{+}$ MC morphologically resembling Peyer's patch microfold cells, with dendritic processes and an associated "lymphocyte pocket." They express transcripts associated with microfold cells, such as *Ccl6, Tnfrsf11b*, and *Gp2* [38]. Microfold mTECs are lost in *Spib*$^{-/-}$ and *Sox8*$^{-/-}$ mice [38], and the differentiation of thymic B cells is perturbed [38,39].

Muscle mTEC:

Myog^{+} MC morphologically resembling and expressing transcripts (*Ckm, Des, Myl1*) associated with skeletal muscle [8,36,38]. Expression of a model antigen in muscle mTECs is sufficient to induce cognate T-cell tolerance [38].

Neuroendocrine mTEC:

Foxa2^{+} *Foxa3*$^{+}$ *Insm1*$^{+}$ *Ascl1*$^{+}$ MC expressing transcripts encoding neuroendocrine markers (*Scg5, Snap25, Chga, Stxbp5l*) and possessing abundant secretory granules [35,38,39]. Under the umbrella of neuroendocrine mTECs, further heterogeneity exists including *Ptf1a*$^{+}$ pancreatic-like, *Cdx2*$^{+}$ enteroendocrine-like, *Pax6*$^{+}$, and *Sox11*$^{+}$ subsets

([38] and our unpublished analysis of data from [38]). Neuroendocrine mTECs are lost in *Foxn1*^{cre} *Insm1*^{flox} and *Foxn1*^{cre} *Ascl1*^{flox} mice, and *Foxn1*^{cre} *Insm1*^{flox} develop autoantibodies against the thyroid and enteroendocrine-cell-rich gastric fundus [39].

Parathyroid mTEC:

Gcm2^{+} MC expressing microscopically detectable parathyroid hormone (PTH) [29,58]. Whether these cells are *bona fide* thymic epithelium or ectopic developmental remnants is controversial and has been debated elsewhere [29,58,59].

Secretory mTEC:

Foxa1^{+}*Spdef*$^{+}$ MC expressing transcripts (*Gabrp, Aqp4, Scgb3a2, Sftpd, Muc5ac, Muc5b*) associated with mixed secretory cell types, including goblet cells, club cells, and alveolar epithelial cells [38].

Thyroid mTEC:

Poorly characterized MC identified as thyroglobulin- and calcitonin-expressing cells by immunofluorescent microscopy [29] More work is needed to determine whether these cells are regular features of the MC compartment.

Tuft mTEC:

Abundant *Pou2f3*$^{+}$ MC expressing markers of tuft cells (IL-25, ChAT, Dclk1), mediate tolerance to tuft-cell-restricted antigens and control the accumulation of type 2 ILCs and NKT cells in the thymus [32,33,60]. Tuft mTECs are lost in *Pou2f3*$^{-/-}$ and *Trpm5*$^{-/-}$ mice [32,33].

OUTSTANDING QUESTIONS BOX

- Precisely which TFs control which mimetic cells?
- How do Aire and lineage-defining TFs integrate, or not, to induce PTAs and mimetic cells?
- Can TF networks be dynamically controlled to output different mimetic cells?
- What makes mTECs permissive to the ectopic action of lineage-defining TFs?
- Are there unique advantages to expressing PTAs in the context of mimetic cells?
- What impact do mimetic cells have beyond selecting the αβ T-cell repertoire?
- What are the immunological sequelae of defects in the various mimetic cell subtypes?
- How does variation in human mimetic cells contribute to autoimmune disease risk?

SIGNIFICANCE

Centuries-old observations of “misplaced” cells in the thymic medulla have recently been unified in the description of mimetic cells, specialized thymic epithelial cells that appropriate the lineage-defining transcription factors of diverse cell types to express peripheral-tissue antigens within the thymus and tolerize maturing T cells. Defects in mimetic cells may play major roles in the pathogenesis of autoimmunity.

HIGHLIGHTS

- Mimetic cells are medullary thymic epithelial cells that mimic diverse extra-thymic cell types
- Lineage-defining transcription factors drive peripheral mimicry in mimetic cells
- Mimetic cells can be necessary and sufficient for antigen-specific T-cell tolerance
- Compartmentalized expression of self-antigens in mimetic cells may explain syndromic manifestations of autoimmunity

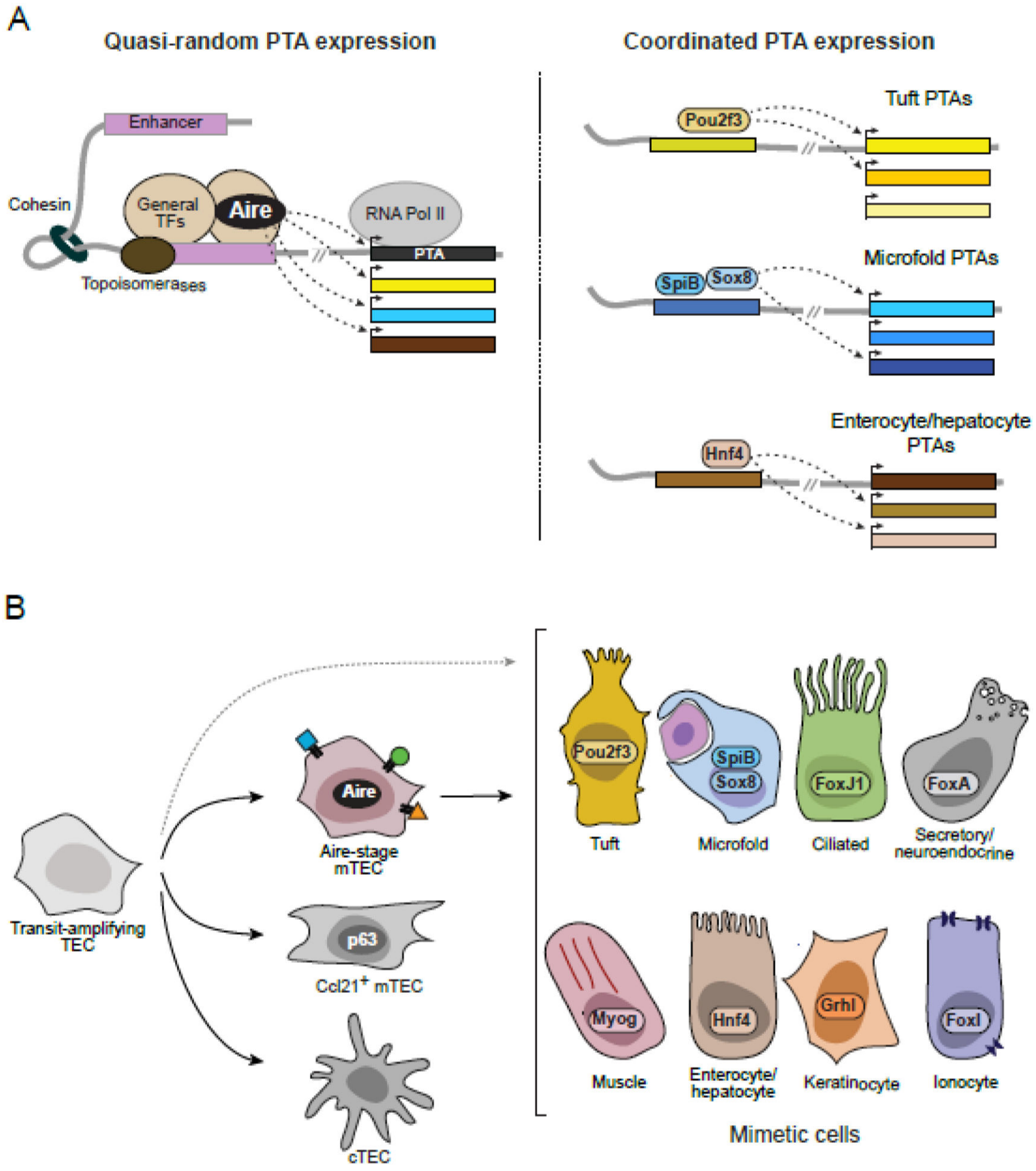

Figure 1 (Key Figure): Model of thymic PTA expression.

(A) Molecular model of PTA induction by Aire (left) and lineage-defining TFs (right). Aire binds promiscuously to mTEC enhancers and promoters and interacts with general transcriptional mechanisms to quasi-randomly activate expression of diverse PTAs [25]. In contrast, lineage-defining TFs bind specifically to cell-type-specific enhancers and drive coordinated expression of biologically coherent sets of PTAs [38]. (B) Cellular model of PTA expression by mTECs. Transit-amplifying TECs give rise to multiple TEC lineages, including cTECs, Ccl21^{+} mTECs, and Aire-stage mTECs [40]. Aire-stage mTECs further differentiate into diverse mimetic cell types characterized by the lineage-defining TFs, chromatin landscapes, and gene expression programs of peripheral cell types [38,39]. Some mimetic cells (i.e., muscle, tuft mTECs) may not necessarily go through an Aire-positive

mTEC stage, as indicated by the dashed line [33,38,39]. Note that Ccl21^{+} mTECs have been called “immature” mTECs, but likely represent a separate mTEC lineage as opposed to an “immature” progenitor [40].

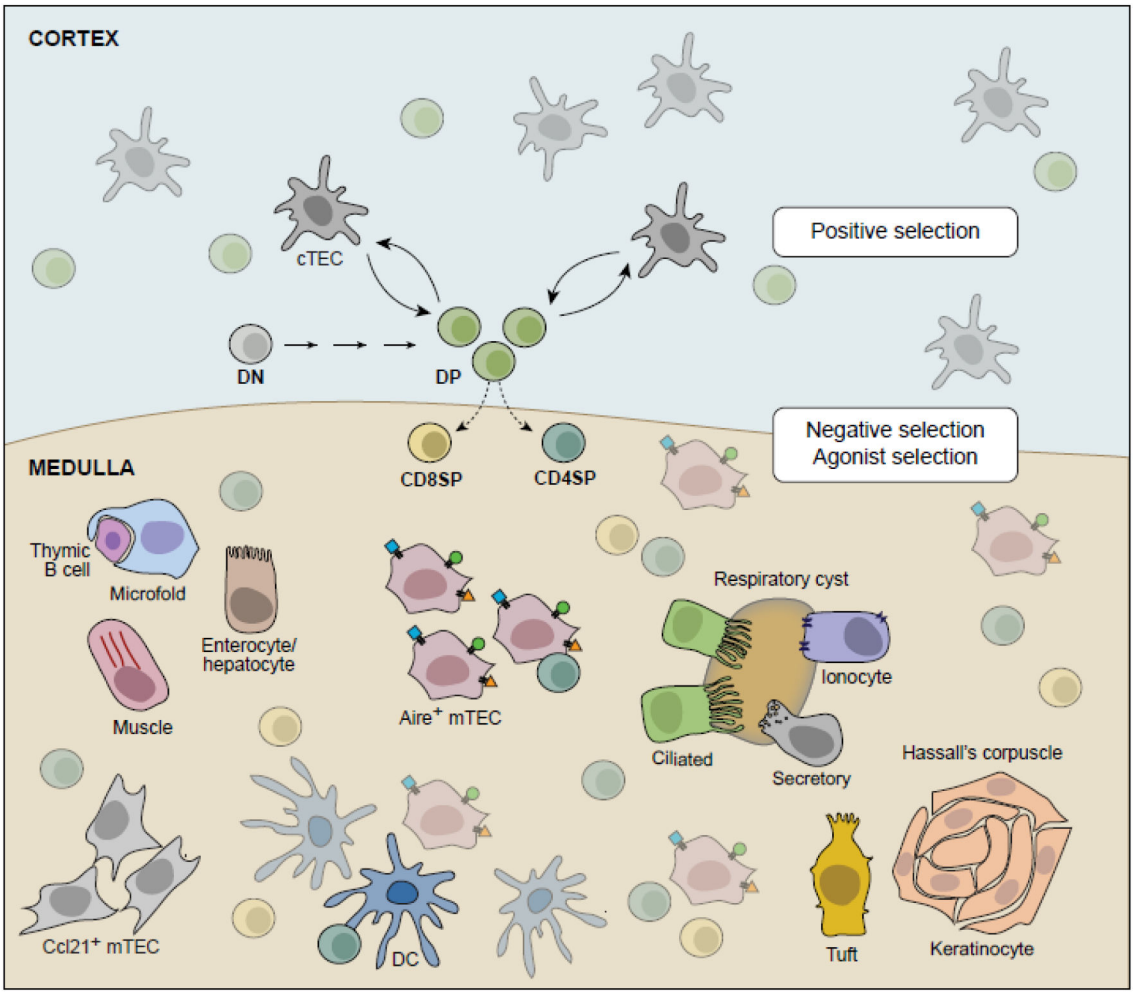

Figure 2: Model of αβ T-cell selection.

Simplified model of αβ T-cell selection. Most thymocytes mature from $CD4^{-}CD8^{-}$ double-negative (DN) precursors into $CD4^{+}CD8^{+}$ double-positive (DP) thymocytes expressing rearranged αβ TCRs. DP thymocytes undergo positive selection for self-MHC molecule restriction through interactions with cTECs; surviving thymocytes become $CD4^{+}$ and $CD8^{+}$ single-positive (SP) thymocytes and undergo negative selection or agonist selection through interactions with thymic APCs in the cortex (ubiquitous antigens) and medulla (PTAs). mTECs, including diverse mimetic cells, provide PTAs for negative and agonist selection, either directly through presentation on MHC molecules or indirectly through antigen transfer to other thymic APCs, including dendritic cells (DCs).