Annual Review of Cell and Developmental Biology

Thymic Mimetic Cells: Ontogeny as Immunology

Daniel A. Michelson[1] and Diane Mathis[2]

[1]Harvard-MIT Program in Health Sciences and Technology, Harvard Medical School, Boston, Massachusetts, USA; email: dam@mit.edu

[2]Department of Immunology, Harvard Medical School, Boston, Massachusetts, USA; email: dm@hms.harvard.edu

Annu. Rev. Cell Dev. Biol. 2024. 40:3.1–3.18

The *Annual Review of Cell and Developmental Biology* is online at cellbio.annualreviews.org

https://doi.org/10.1146/annurev-cellbio-112122-023316

Keywords

thymus, mTEC, mimetic cell, T cell, tolerance, autoimmunity

Abstract

Medullary thymic epithelial cells (mTECs) generate immunological self-tolerance by ectopically expressing peripheral-tissue antigens (PTAs) within the thymus to preview the peripheral self to maturing T cells. Recent work, drawing inspiration from old histological observations, has shown that subtypes of mTECs, collectively termed mimetic cells, co-opt developmental programs from throughout the organism to express biologically coherent groups of PTAs. Here, we review key aspects of mimetic cells, especially as they relate to the larger contexts of molecular, cellular, developmental, and evolutionary biology. We highlight lineage-defining transcription factors as key regulators of mimetic cells and speculate as to what other factors, including Aire and the chromatin potential of mTECs, permit mimetic cell differentiation and function. Last, we consider what mimetic cells can teach us about not only the thymus but also other tissues.

Contents

INTRODUCTION

T cells, essential components of the vertebrate adaptive immune system, are so named because they differentiate in the thymus, a shield-shaped organ that sits in the chest just above the heart in mice and humans. For a recent review of thymic T cell differentiation, readers are referred to Ashby & Hogquist (2024). Within the thymus, hematopoietic progenitors commit to the T cell lineage, generate their trademark antigen-specific T cell receptors (TCRs), become restricted to recognize antigens only within the context of major histocompatibility complex (MHC) molecules, and learn to discriminate between self- and nonself-antigens. This last process, otherwise known as central or thymic tolerance, involves the negative selection of T cells, wherein T cells bearing autoreactive TCRs are clonally deleted from the mature repertoire, as well as agonist selection, wherein T cells with autoreactive TCRs are diverted into a tolerogenic fate, such as the regulatory T cell lineage. T cells that successfully pass central tolerance without being deleted or diverted continue differentiating into conventional T cells and exit the thymus into the rest of the body, ready to protect the host from foreign antigens while sparing it from autoimmunity.

Negative selection: process by which autoreactive T cells are deleted in the thymus

Medullary thymic epithelial cell (mTEC): specialized cell type responsible for negative selection and Treg generation

Peripheral-tissue antigen (PTA): a protein whose expression is specific to extrathymic tissues

Autoimmune regulator (Aire): transcriptional regulator expressed in mTECs that upregulates thousands of PTAs

Medullary thymic epithelial cells (mTECs) play a unique role in thymic tolerance by expressing the great majority of self-antigens ectopically within the thymus (reviewed in Klein et al. 2014). This includes ubiquitous antigens, such as actin or tubulin, as well as antigens whose expression is normally quite restricted to just one or a few tissues, such as insulin, mucins, or myelin. Collectively, the antigens within the second class are known as peripheral-tissue antigens (PTAs). PTA expression by mTECs previews the peripheral self to maturing T cells while they are still in the thymus, allowing for the deletion or diversion of self-reactive cells before they can cause harm.

How a single cell type, the mTEC, manages to express so many self-antigens, many of whose expression is normally tightly controlled within particular cell types in the periphery, has been a source of long-standing intrigue. Two decades ago, a transcriptional regulator called autoimmune regulator (Aire) was shown to play an important role in self-antigen expression, as its ablation diminishes the expression of a large repertoire of PTAs within mTECs, and mice and humans with *Aire/AIRE* mutations develop autoimmunity against Aire-induced self-antigens (Aaltonen et al. 1997, Anderson et al. 2002, Nagamine et al. 1997). Subsequent mechanistic studies found that Aire acts stochastically, probabilistically, or quasi-randomly within individual cells to induce diverse and

disparate genes without any particular structuring logic (Brennecke et al. 2015, Derbinski et al. 2008, Meredith et al. 2015, Villasenor et al. 2008). Given this pattern of gene activation, it has been proposed that Aire utilizes noncanonical transcriptional mechanisms—including DNA-damage-induced transcriptional activation, promotion of RNA polymerase II pause release, and global activation of enhancers and enhancer-promoter looping—to promote PTA expression in mTECs (Abramson et al. 2010; Bansal et al. 2017, 2021; Giraud et al. 2012; Guha et al. 2017; Sansom et al. 2014; Yoshida et al. 2015). Still, questions have lingered about precisely how Aire selects its target genes and how essential Aire is to PTA expression.

Mimetic cell: mTEC that mimics the chromatin, transcriptional, and phenotypic state of an extrathymic cell type

More recently, a second mechanism of PTA expression was discovered, involving subtypes of mTECs that mimic the chromatin, transcriptomic, and phenotypic states of diverse extrathymic cell types, such as keratinocytes, enterocytes, and ciliated cells (Michelson et al. 2022b). These mTECs were collectively termed mimetic cells. In contrast to Aire-driven PTA expression, mimetic cells show a clear organization of PTA expression according to a cell-type logic, thus upending the prevailing notion that PTA expression by mTECs is entirely stochastic or quasi-random. Importantly, the existence of mimetic cells does not negate the well-established importance of Aire in PTA expression; rather, the two mechanisms appear to coexist in separate subtypes of mTECs, and likely interrelate over the course of mTEC differentiation.

In this review, we begin with an overview of the history and current knowledge of mimetic cells. Because the immunological roles of PTA expression, Aire, and mimetic cells have been reviewed elsewhere, we provide only a brief overview and refer the interested reader to those publications (Kaiser et al. 2022, Klein et al. 2014, Michelson & Mathis 2022). We focus instead on situating mimetic cells within the larger frameworks of molecular, cellular, developmental, and evolutionary biology, exploring what insights other areas of biology might have for the thymus and, in turn, what insights the thymus might have for these other contexts. From there, we discuss translational and therapeutic potentialities of mimetic cells. Finally, we offer suggestions for future work in this area.

HISTORY OF MIMETIC CELLS

Although the term mimetic cell has only recently come into use, scientists have observed cells with features of diverse peripheral cell types misplaced within the thymic medulla by light microscopy since at least the mid-nineteenth century. For instance, Robert Remak (1855, p. 124), a Polish embryologist, described the "wimperblasen der thymus" (eyelashes of the thymus):

> A very striking fact is that I noticed. . .the occurrence of pedunculated eyelashes attached to the thymus lobes in young cats. These ciliated vesicles resemble those which I have already observed earlier in the mesogastrium in frog and the mesometrium in rabbits. The wall consists of a firm layer of connective tissue and an epithelium covered with vibrating eyelashes.

Remak noted that the ciliated cells of the thymus resembled ciliated epithelium from elsewhere in the body, though their appearance in the thymus was "striking" given the absence of a discernable biological function there. These cells likely represent the same ciliated cells described in the thymus by immunofluorescence and electron microscopy more than a century later (Farr et al. 2002) and then by single-cell RNA sequencing (scRNA-seq) and single-cell assay for transposase accessible chromatin and sequencing (scATAC-seq) two decades after that (Dhalla et al. 2020, Michelson et al. 2022b). Other such examples of histologically described mimetic cells include the cornified, skin-like Hassall's corpuscles, eponymously named for Arthur Hill Hassall (1846), and the thymic "myoidzellen," striated cells resembling skeletal muscle described by Sigmund Mayer (1888).

A major barrier to understanding the functions of mimetic cells at the time of their discovery was a lack of understanding of the function of the thymus itself. Hassall (1846, p. 9), for instance, believed the thymus to be "an organ of nutrition, adapted to the special exigencies of early life." Until the 1960s, the thymus was widely considered vestigial, with such leading scientists as Peter Medawar (1963, p. 70) proclaiming as late as the 1960s that "we shall come to regard the presence of lymphocytes in the thymus as an evolutionary accident of no very great significance." Without a coherent picture of thymus biology, there was no framework within which to understand mimetic cells and their strange peripheral mimicry. Herbert Watney (1882, p. 1094), a British physician, expressed his puzzlement about Remak's and his own studies on thymic ciliated cells:

> The ciliated cysts can therefore be shown to arise from concentric corpuscles; and these, as we have seen above, always primarily from connective-tissue-corpuscles. We therefore come to the conclusion *that the connective-tissue-corpuscles can undergo certain changes, which finally transform them into ciliated epithelium*. There is no doubt a considerable a priori improbability in this statement, as we should expect ciliated epithelium to be found in association with a higher organization, and not as one of the methods of involution or degeneration of the tissues; and further, the view stated above is incompatible with those which have been so long held concerning the respective functions of mesoblast and hypoblast. There seems, however, no other conclusion left to us.

Even within the scientific schema of the nineteenth century, Watney could appreciate that ciliated cells were developmentally out of place in the thymus. But, lacking modern molecular tools, he could not interrogate how and why they nevertheless appeared, and, as a result, these early observations laid dormant for over a century.

In the 1980s and 1990s, with the importance of the thymus in T cell tolerance better appreciated, several studies discovered PTA expression within the thymic medulla using immunofluorescence microscopy and reverse transcription and polymerase chain reaction (Antonia et al. 1995, Jolicoeur et al. 1994, Kirchner et al. 1988). Several molecular models for this ectopic gene expression were proposed at the time, including one that postulated a link with the various histologically distinct cell types described microscopically by Remak, Hassall, and others (Farr & Rudensky 1998). However, Aire was implicated in PTA expression a short while later, and a stream of subsequent studies confirming Aire's importance, coupled with a dearth of additional studies on mimetic cells, eventually led to a general consensus that Aire was the key regulator of central tolerance (Danso-Abeam et al. 2013, Devoss et al. 2006, Gavanescu et al. 2007, Kuroda et al. 2005, Liston et al. 2003).

MIMETIC CELLS IN THE TWENTY-FIRST CENTURY

In the last few years, single-cell genomics have enabled the reemergence and elevation of mimetic cells alongside Aire as important features of thymic tolerance. Key waypoints have included the discovery and rediscovery of individual mimetic cell subtypes by single-cell sequencing, definition of mimetic cells in aggregate, appreciation of the breadth of mimetic cells, identification of lineage-defining transcription factors (TFs) as central to their differentiation, and association of mimetic cells with T cell tolerance. Most of the studies referred to here have been conducted in mice, though several focused on mimetic cells in other organisms are also highlighted.

Tuft mTECs were among the first mimetic cells to be molecularly defined owing to their relative abundance in the murine thymus. As the cellular throughput of single-cell sequencing technologies increased, two scRNA-seq studies simultaneously discovered a distinct subset of mTECs expressing molecules (e.g., IL-25, choline acetyltransferase, TRPM5) associated with tuft cells, type 2 immune sentinels found in the gut, lung, and other mucosal epithelia (Bornstein et al. 2018, Miller et al. 2018). As with other mimetic cells subsequently discovered, tuft mTECs previously had been observed histologically (Panneck et al. 2014), but little was known

about their molecular biology or cellular function. Together, the two studies described several key features of tuft mTECs that presaged general principles of mimetic cells, including their dependence on a lineage-defining TF (Pou2f3) for differentiation, their ability to induce T cell tolerance to a tuft cell antigen (IL-25), and their influence on other cells within the thymic milieu. Several subsequent studies using scRNA-seq also found mimetic cell subsets, such as neuroendocrine or ciliated mTECs (Baran-Gale et al. 2020, Dhalla et al. 2020, Wells et al. 2020), though in-depth characterization was not performed.

Another important advance came with the application of scATAC-seq to mTECs, which revealed that each subset of mimetic cells possessed the characteristic motifs of lineage-defining TFs from peripheral tissues within their accessible chromatin (Michelson et al. 2022b). These data at once suggested a molecular mechanism for the existence of mimetic cells, offered a TF-centric framework for organizing mimetic cells, and suggested a new mechanism beyond Aire by which mTECs might produce PTAs for T cell tolerance. The term mimetic cells was proposed for the first time to label these peripheral cell–mimicking mTECs in aggregate. Finally, mimetic cells were shown to be enriched within the Pdpn^{-}CD104^{-}MHCII^{lo} mTEC compartment, paving the way for future studies focused on mimetic cells as a collective entity.

Several key principles of mimetic cells have been established to date, summarized here, and explored at length in turn below. First, lineage-defining TFs are central to the differentiation and PTA expression of mimetic cells. Second, mimetic cells retain their mTEC identity but adopt major chromatin, transcriptional, and phenotypic features of their peripheral counterparts. Third, Aire-expressing mTECs and mimetic cells are distinct, though developmentally related, entities. Fourth, mimetic cells are conserved across evolutionary time. Fifth, and finally, defects in mimetic cells cause autoimmunity, and the manipulation of mimetic cells may be of corresponding benefit in autoimmunity, cancer, and other disease states.

TRANSCRIPTION FACTORS AND MIMETIC CELLS

The generation of mimetic cells is a fundamentally TF-driven phenomenon. This notion bears repeating because, while cell-type analogies between mimetic cells and specific peripheral cell types are useful as a readily interpretable shorthand, mimetic cells are fundamentally TF-driven. Ciliated mTECs, for instance, might immediately evoke airway ciliated cells, with which many biologists are familiar, but numerous other tissues also contain ciliated cells, including the choroid plexus, fallopian tubes, and testes, each with its own context-specific subtleties. Ciliated mTECs do not map perfectly onto any of these contexts but instead represent a sort of distilled, primitive ciliated cell. The common feature of ciliated mTECs and ciliated cells in other tissues appears to be the lineage-defining TFs that drive ciliated cell–specific gene expression. The primacy of TFs in driving mimetic cell gene expression explains why, in every case examined so far, mimetic cells maintain a primarily thymic transcriptomic identity and layer on their mimetic cell gene programs as minor (albeit substantial) components (Michelson et al. 2022b, 2023). TF primacy also explains why, in some cases, mimetic cells appear to straddle multiple peripheral cell–type identities, as with enterocyte-hepatocyte (entero-hepato) mTECs that mirror aspects of both gut enterocytes and liver hepatocytes (Michelson et al. 2023).

Every mimetic cell characterized thus far possesses one or several lineage-defining TF(s) marking its chromatin and driving its differentiation and associated PTA expression (**Figure 1**). For example, tuft mTECs require the tuft cell–specific TF Pou2f3 (Bornstein et al. 2018, Miller et al. 2018), microfold mTECs the microfold cell (M cell)–specific TFs SpiB and Sox8 (Givony et al. 2023, Michelson et al. 2022b), entero-hepato mTECs the gut and liver TF Hnf4γ (Michelson et al. 2023), and neuroendocrine mTECs the neuroendocrine TFs Insm1 and Ascl1 (Givony et al. 2023, Tao et al. 2023). Additional mimetic cells have been proposed to rely on other lineage-defining

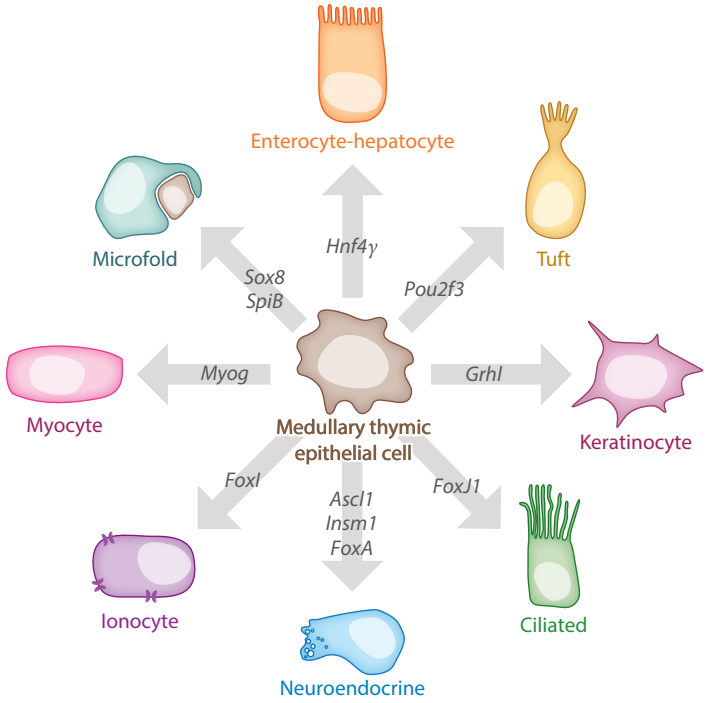

Figure 1

Mimetic cells and their controlling TFs. Mimetic cells differentiate from mTEC progenitors via the action of diverse transcription factors, including muscle via Myog; microfold via Sox8 and SpiB; enterocyte-hepatocyte via Hnf4γ; tuft via Pou2f3; keratinocyte via Grhl1; ciliated via FoxJ1; neuroendocrine via Ascl1, Insm1, and FoxA family TFs; and ionocyte via FoxI family TFs. Abbreviations: mTEC, medullary thymic epithelial cell; TFs, transcription factors.

TFs, such as keratinocyte mTECs on grainyhead-like (Grhl) family TFs, muscle mTECs on myogenin (Myog), and ionocyte mTECs on forkhead-box I (FoxI) family TFs, based on motif analysis of accessible chromatin, chromatin binding assays, and analogy to peripheral tissues, though we still await definitive evidence from knockout mouse models for these factors (Michelson et al. 2022b).

Substantial evidence has thus accumulated indicating that lineage-defining TFs are necessary for mimetic cells. A recent study tackled a related question, that of whether lineage-defining TFs are also sufficient to produce mimetic cells (Tao et al. 2023). The authors developed a mouse model that conditionally overexpressed Insm1, the lineage-defining TF for neuroendocrine mTECs, within thymic epithelial cells (TECs). Insm1-overexpressing mTECs showed enhanced expression of neuroendocrine-associated transcripts and a few Aire-associated transcripts but no transcripts associated with other mimetic cells such as entero-hepato mTECs, indicating that the enhanced expression of a lineage-defining TF was sufficient to enhance expression of its downstream PTAs.

How, molecularly, do lineage-defining TFs exert their effects in mTECs to permit the differentiation of mimetic lineages? The factor(s) upregulating lineage-defining TFs in the first place remains unclear but may include extrinsic signaling cues, the action of other TFs, and quasi-random upregulation by Aire. The mode of upregulation may also differ between lineage-defining TFs: In microfold mTECs, for instance, SpiB deletion downregulates *Sox8*, but Sox8 deletion does not affect expression of *Spib*. At a genomic level, several lineage-defining TFs directly bind

to mTEC chromatin at cognate motif–containing open chromatin regions (OCRs), including Pou2f3, Grhl1, Hnf4α, Hnf4γ, and Insm1 (Michelson et al. 2022b, 2023; Tao et al. 2023). Whether these TFs are pioneering (binding at and opening compacted chromatin) in the manner often associated with lineage-defining TFs has not been directly established, but many OCRs bound by lineage-defining TFs are closed in most mTECs and open only within their respective mimetic cells, indicating coincident TF binding and nucleosomal decompaction and suggesting that at least some TFs may directly open chromatin in mimetic cells (Michelson et al. 2022b).

A recent study explored the molecular influences of lineage-defining TFs on the epigenetic state of mTECs by focusing on Hnf4α and Hnf4γ (together, Hnf4), which control entero-hepato mTECs (Michelson et al. 2023). Hnf4 promoted the deposition of H3K27ac and H3K4me1, histone marks associated with enhancer activation, at entero-hepato enhancers. An earlier study of intestinal epithelial cells (IECs) found a similar action of Hnf4 at IEC enhancers, suggesting that the molecular activity and targets of Hnf4 are conserved in the two contexts (Chen et al. 2019). Hnf4 also induced the redistribution of CTCF to entero-hepato OCRs, suggesting that lineage-defining TFs might influence mimetic cell genome structure, reminiscent of the influence of Aire on genome structure in Aire-expressing mTECs (Bansal et al. 2021). Conversely, Hnf4 had little effect on promoters or gene bodies, with no discernable influence on H3K4me3, H3K36me3, or promoter-proximal H3K27ac. This result comported with data from Insm1 conditional-mutant mice showing that Insm1 binding at gene promoters was not predictive of the transcriptional dysregulation of the same genes upon deletion of Insm1 in mTECs (Tao et al. 2023). Finally, Hnf4 had no effect on Polycomb-mediated repression as measured by H3K27me3 levels.

Thus, the emerging picture shows that lineage-defining TFs play central roles in determining mimetic cell identity. They are both necessary and sufficient for the differentiation of mimetic cells and PTA expression. Mechanistically, they drive mimetic cell biology via the opening of mTEC chromatin, deposition of H3K27ac and H3K4me1 at distal regulatory elements, and redistribution of CTCF; however, they do not substantially influence promoter-proximal or Polycomb-mediated regulatory mechanisms. As additional models perturbing mimetic cells become available, these results should be extended to other lineage-defining TFs and mimetic cells to test their generality.

CHROMATIN POTENTIAL OF MEDULLARY THYMIC EPITHELIAL CELLS

That lineage-defining TFs can drive mimicry of diverse cellular lineages within the thymus is itself unexpected given the careful spatiotemporal regulation of TF activity during development. There may be special properties of mTECs that permit the ectopic activity of lineage-defining TFs in mimetic cells. Several proposed mechanisms are illustrated in **Figure 2** and explored further below.

The thymic epithelium derives from the endodermal germ layer of the early embryo, specifically the third pharyngeal pouch endoderm in mice and humans. Thymic development has been reviewed in depth elsewhere (Rodewald 2008), and only elements potentially relevant to mimetic cell biology are summarized here. By embryonic day 11.5 (E11.5) in mice, TEC precursors express the lineage-defining TF for thymic epithelium, FoxN1. Shortly thereafter, hematopoietic T cell precursors colonize the thymus and provide signals important for corticomedullary differentiation. Single Foxn1^{+} TECs from E12 mice can give rise to both cortical thymic epithelial cells (cTECs) and mTECs when grafted into recipient mice, though individual TEC progenitors are often biased towards cTEC or mTEC lineages (Bleul et al. 2006, Mayer et al. 2016, Nusser et al. 2022, Rossi et al. 2006).

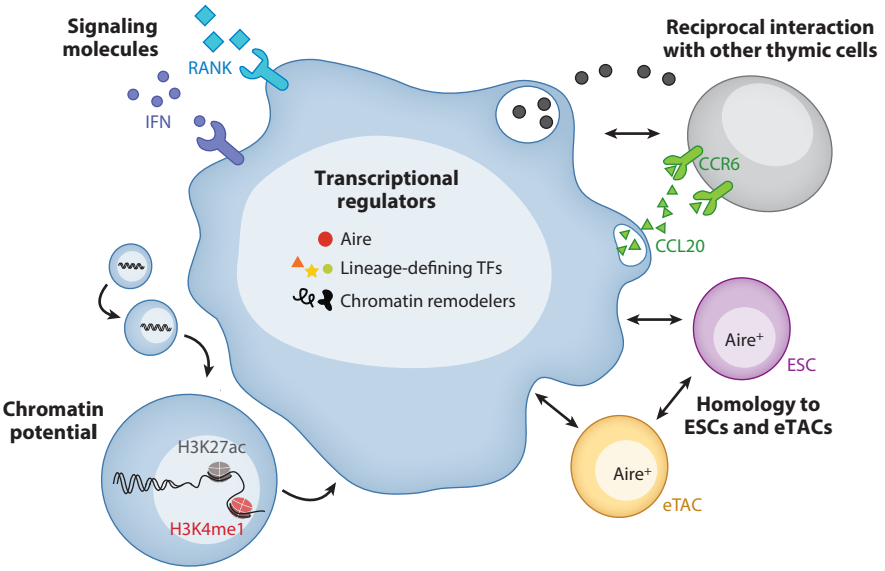

Figure 2

Potential mechanisms enabling the differentiation of mimetic cells. Intrinsic factors may include the action of Aire, lineage-defining TFs, and chromatin remodelers on the mTEC genome, which itself inhabits a unique epigenetic state. Extrinsic factors may include general signals from the thymic milieu such as RANK and IFN as well as specific interactions with other thymic cells. Comparison of Aire's role in thymic and extrathymic contexts may help define its role in mimetic cell differentiation. Abbreviations: Aire, autoimmune regulator; ESCs, embryonic stem cells; eTACs, extrathymic Aire-expressing cells; IFN, interferon; mTEC, medullary thymic epithelial cell; TFs, transcription factors.

Though no studies have lineage traced uncommitted TEC progenitors all the way to mimetic cells, several insights have been gleaned from more limited studies. One straightforward conclusion is that mimetic cells are derived from mTECs but not cTECs given the simple observation that all mimetic cells studied to date localize to the medulla of the thymus (Michelson et al. 2022b). The one exception is parathyroid mimetic cells, which have been observed under the thymic capsule in the cortex, but which may reflect anatomic missegregation of the third pharyngeal pouch rather than true TEC-derived mimetic cells (Gunther et al. 2000, Liu et al. 2010). Interestingly, blastocyst chimera experiments have shown that individual medullary islets are clonally derived from individual mTEC precursors (Rodewald et al. 2001). Given that some types of mimetic cells are asymmetrically distributed throughout the thymus, one can imagine that individual clonal TEC precursors might be biased not just towards cTEC versus mTEC lineages but also towards particular mimetic cells.

Lineage analysis using Cre-based reporter mice has shown that the majority of mimetic cells, though not all, have previously expressed Aire and casein beta, a milk protein induced in Aire-expressing mTECs (Givony et al. 2023, Michelson et al. 2022b). These data underscore that Aire-expressing mTECs are a major precursor for mimetic cells, though the exact role of Aire in mimetic cell differentiation remains to be clarified. Experiments with Cre-based reporter mice have suggested that mimetic cell identities are fairly stable once formed since genetically labeled mimetic cells (tuft, ciliated, and muscle mTECs) retain coexpression of lineage-specific molecules (Dclk1, acetylated tubulin, and desmin, respectively) over successive weeks and do not show evidence of conversion into other mimetic cell lineages (Michelson et al. 2022b; D.A. Michelson & D. Mathis, unpublished data). These data are consistent with the long-held notion that post-Aire

mTECs, which are enriched for mimetic cells, occupy a state of terminal differentiation (Metzger et al. 2013, Nishikawa et al. 2014).

Spatially, mimetic cells distribute in multiple patterns. Some localize to microstructures with substantial internal organization and complexity: For instance, respiratory cysts are lined by ciliated cells that possess apico-basal polarity and are interspersed with cells with mucus-producing morphologies (Farr et al. 2002, Michelson et al. 2022b). On the other hand, other mimetic cells, such as microfold, entero-hepato, or muscle mTECs, are distributed apparently randomly, without any clear relation to other medullary landmarks (Dhalla et al. 2020; Michelson et al. 2022b, 2023). Still, a random distribution does not mean that these mimetic cells lack spatial organization; on the contrary, for instance, microfold mTECs strongly associate with thymic B cells and certain thymic antigen-presenting cells (APCs) in a Ccr6-dependent manner (Givony et al. 2023). Still other mimetic cells may appear to distribute randomly but reveal themselves to associate with particular mimetic cell subtypes when appropriately co-stained, as is the case for tuft mTECs and Hassall's corpuscles (Miller et al. 2018). Systematic and high-dimensional analysis of mimetic cell localization and hematopoietic cell colocalization patterns should provide a much more conclusive perspective on the spatial distribution of mimetic cells.

Assimilating these data, are there features of thymic development and mTEC differentiation that might explain why TECs can produce peripheral mimicry in a manner unprecedented elsewhere in the body? Both cTECs and immature ($Ccl21^+$) mTECs have monomorphic gene signatures and play clearly delineated roles in tissue biology, suggesting that the epithelium of the thymus is not intrinsically different from that of other tissues. Instead, Aire-expressing mTECs and mimetic cells likely have unique cellular features that enable peripheral mimicry. Recent studies of the chromatin states of mTEC subsets support this notion, showing that Aire-expressing mTECs dramatically remodel their accessible chromatin compared with their cellular precursors (Koh et al. 2018, Michelson et al. 2022b). TF-driven programs in mimetic cells may be thought of as pure effects of TFs enabled by a permissive cellular context, similar to those defined in a recent, multiplexed TF atlas investigating the effects of ectopic TF expression in embryonic stem cells (ESCs) (Joung et al. 2023). Several programs homologous to those of mimetic cells were uncovered in that study, such as an Hnf4-driven endodermal program and a Grhl-driven epidermal program. Altogether, mimetic cells likely reflect the activity of diverse TFs within an unusually permissive chromatin state generated by Aire-expressing mTECs.

MIMETIC CELLS AND AIRE

It would be reasonable to hypothesize that because mimetic cells differentiate largely from Aire-expressing mTECs, Aire itself should have a central role in producing mimetic cells. However, the data present a more complicated story. Aire does have an undeniably important role in mimetic cell differentiation as multiple mimetic cell types, such as neuroendocrine, ciliated, and microfold mTECs, are reduced in frequency and number in the genetic absence of Aire, and the activity of several lineage-defining TFs is diminished in the molecular absence of Aire (Michelson et al. 2022b). However, Aire is not strictly required for mimetic cells as deletion of Aire does not result in the frank absence of any mimetic cell type (Michelson et al. 2022b). Furthermore, muscle mTECs and a subset of tuft mTECs differentiate from pre-Aire progenitors according to lineage tracing (Givony et al. 2023, Michelson et al. 2022b, Miller et al. 2018); another mechanism is required to explain the appearance of these mimetic cells in the absence of a developmental history of Aire expression.

Several studies have identified regulators of PTA expression beyond Aire, though in most cases their specific effects on mimetic cells have not been examined. For instance, studies using mouse genetic models have presented evidence that Mbd1 (a DNA methylation reader), Fezf2 (a

Extrathymic Aire-expressing cell (eTAC): a heterogenous set of hematopoietically derived cells involved in peripheral tolerance

brain-expressed TF), and Chd4 (a chromatin remodeler) regulate PTA expression (Takaba et al. 2015, Tomofuji et al. 2020, Waterfield et al. 2014). These factors may regulate PTA expression via effects on mimetic cell differentiation, though this remains speculative for now. Additionally, mass spectrometry of Aire-associated proteins and RNA interference of TECs have identified numerous candidate regulators of PTA expression, generally grouping into nuclear transport, chromatin binding, transcription-associated, and RNA-processing categories (Abramson et al. 2010, Giraud et al. 2014). Some of these factors may exert additive or synergistic effects with Aire to drive mimetic cells. Ultimately, knockout models—preferably temporally controlled—coupled with the careful quantitation of resulting mimetic cell compartments should reveal the contributions of these factors in producing mimetic cells.

The best comparator cell type for Aire-expressing mTECs, and the one that might yield the most insight into Aire's influence on mimetic potential, may be the ESC, which can develop from a single totipotent precursor to give rise to all tissues of the body. Intriguingly, Aire is also expressed in ESCs, a fact made evident when Aire-Cre mice were first generated and all tissues in the adult mouse were found to report Cre activity (Nishikawa et al. 2010). In ESCs, Aire promotes stem cell proliferation and enhances the efficiency of blastocyst formation (Gu et al. 2010, 2017), though it is not strictly required for embryonic development as the blastocysts that do form are structurally normal and mice and humans with *Aire/AIRE*-null mutations are viable. Though circumstantial, the presence of Aire in both ESCs and mTECs hints at a common role for Aire in increasing permissiveness to multiple cellular fates. As in blastocyst formation from ESCs during early development, Aire may promote the efficiency of mimetic cell differentiation from mTEC precursors without being strictly required.

Notably, the embryo is not the only place that Aire expression has been found. Extrathymic Aire-expressing cells (eTACs) are a heterogeneous group of Aire-expressing, hematopoietic-lineage cells with proposed roles in peripheral, maternal-fetal, and microbial tolerance as well as fungal defense (Akagbosu et al. 2022; Dobes et al. 2022; Gardner et al. 2008, 2013; Gillis-Buck et al. 2021; Kedmi et al. 2022; Lyu et al. 2022; Wang et al. 2021; Yamano et al. 2019). The field is rapidly evolving, with a still unsettled nomenclature that includes eTACs, Aire$^+$ ILC3s, Thetis cells, and Janus cells, and we refer the interested reader to recent reviews of this area (Abramson et al. 2024, Brown & Rudensky 2023, Gardner & Liston 2022). Here, we focus on insights into Aire biology from eTACs. Germ line deletion of Aire followed by transcriptional profiling of Aire$^+$ lymph node cells has found a few hundred Aire-induced genes in eTACs, with only a handful being PTAs and relatively little overlap with Aire-induced genes in mTECs (Gardner et al. 2008, Yamano et al. 2019). The deletion of Aire specifically in RORγ^+ cells during fungal infection impaired the expression of inflammatory cytokines and costimulatory molecules but again had relatively little effect on PTA expression (Dobes et al. 2022). Finally, deletion of Aire in RORγ^+ APCs did not impair cognate RORγ^+ regulatory T cell (Treg) differentiation even though the RORγ^+ APCs themselves were required, suggesting that Aire may be just one of several molecular regulators of a cellular spectrum that includes both eTACs and nonAire-expressing tolerogenic APCs (Akagbosu et al. 2022, Kedmi et al. 2022, Lyu et al. 2022). Thus, while Aire has been proposed to induce PTAs in eTACs to promote extrathymic immunological tolerance, a natural hypothesis given its role within the thymus, the efficacy of Aire in inducing PTAs in eTACs is actually quite limited compared with that of mTECs. Instead, the major commonalities between Aire-expressing mTECs and eTACs seem to be their high expression of MHC class II and costimulatory molecules and their (admittedly diverse) roles in immunological tolerance. We suggest that Aire in eTACs may function more analogously to its role in mimetic cells and the early embryo, conditioning eTACs to upregulate lineage-specific and antigen presentation programs rather than directly driving the expression of PTAs.

Insight may also be gained from the extrinsic signals that induce Aire in the thymus and the periphery. RANK signaling in mTECs stimulates the differentiation of Aire-expressing mTECs and certain mimetic cells (Givony et al. 2023, Metzger et al. 2013, Rossi et al. 2007, Wells et al. 2020). RANK signaling is also required for the accumulation of eTACs (Wang et al. 2021, Yamano et al. 2019), pointing to the central role of this pathway in the regulation of Aire. There may also be a link between Aire and interferon (IFN) signaling through interferon regulatory factor 8 (IRF8): IRF8 induces Aire expression in the thymus (Herzig et al. 2017); Aire expression correlates with an IRF8-driven antigen presentation program (Michelson et al. 2022a,b; Morimoto et al. 2022); and RORγ$^+$ eTACs show enrichment of IRF8 motifs within their accessible chromatin (Akagbosu et al. 2022). Additional work on signals inducing Aire is likely to yield a clearer overall picture of Aire's conserved intra- and extrathymic functions, with cascading insights into its role in producing mimetic cells.

MIMETIC CELLS ACROSS EVOLUTIONARY TIME

To date, most work on mimetic cells has been done in the mouse system, but mimetic cells themselves appear to be well conserved in the adaptive immune system across evolutionary time. Already in the late 1800s, Watney (1882), the British physician who puzzled over the origins of Hassall's corpuscles in the dog thymus, was extending his observations to humans, hedgehogs, cats, lambs, calves, oxen, alpine marmots, bats, guinea pigs, rabbits, pelicans, turkeys, pigeons, chicks, alligators, snakes, tortoises, frogs, axolotls, cod fish, and ray fish. Recent work using single-cell genomics has again updated historical microscopic observations with high-dimensional molecular correlates.

A recent single-cell transcriptomic study of thymopoiesis in zebrafish captured multiple mimetic cell types, providing evidence that mimetic cells are present even in species that diverged from mammals early after the evolution of adaptive immunity (Rubin et al. 2022). Some zebrafish mimetic cells could be annotated based on their similarity to prior analyses in mice, including neuroendocrine and tuft mTECs, whereas others did not map closely onto a single mouse or human analog, such as TECs expressing high levels of enzymes that produce fatty acids. One major challenge of analyzing mimetic cells in zebrafish is a dearth of antibodies relative to mice or humans with which to specifically enrich TEC subpopulations by flow cytometry. The generation of antibodies against zebrafish mimetic cell markers would facilitate a closer study of mimetic cells in humans. Similarly, the development of transgenic zebrafish lines to fluorescently label mimetic cells would enable the easy enrichment of mimetic cells and could be combined with the unique ability to generate optically transparent zebrafish to allow for live imaging of mimetic cells in situ.

Another set of single-cell transcriptomic studies has demonstrated that humans also harbor mimetic cells within their thymuses. One study conducted under the aegis of the Human Cell Atlas reported the presence of mimetic cells analogous to keratinocyte, tuft, neuroendocrine, and muscle mTECs (Park et al. 2020). This study also performed RNA in situ hybridization to verify the presence of muscle mTECs within the thymic medulla. A second study reported the presence of tuft, ionocyte, neuroendocrine, keratinocyte, ciliated, and myelin-producing mTECs in human thymuses and further used immunohistochemistry to localize these subtypes within the thymic medulla, including many in or near Hassall's corpuscles (Bautista et al. 2021). The data thus far suggest that the bulk of mimetic cell types found in mice are also present in humans, though we await focused studies of mimetic cells in humans to more comprehensively understand the similarities and differences between the two species. Finally, both the zebrafish and human studies, though not perfectly quantitative, have suggested mimetic cell subtype abundances that qualitatively deviate from those seen in mice. Further work should address whether intraindividual, intraspecific, and/or other differences drive variation in mimetic cell frequency.

LEARNING PERIPHERAL BIOLOGY FROM MIMETIC CELLS

Because the biology of mimetic cells draws so heavily on biological circuits from throughout the organism, the study of mimetic cells offers insight into not just thymus biology but also the biology of peripheral (extrathymic) tissues. In its original conception, PTA expression within the thymus served as a mirror of the peripheral self. The biological coherence of mimetic cells offers the opportunity to reflect that mirror back onto peripheral tissues, revealing new biology in the process.

This principle was illustrated by studying Hnf4 TFs and their roles in mimetic cells (Michelson et al. 2023). Hnf4 TFs were required for entero-hepato mTEC differentiation, consistent with the known importance of Hnf4 TFs in the differentiation of true gut and liver epithelium. Unexpectedly, one Hnf4 family member, Hnf4γ, was also required for the normal differentiation of microfold mTECs. Microfold mTECs resemble M cells, specialized epithelial cells that line the surface of Peyer's patches and other mucosa-associated lymphoid tissues and sample antigens from the outside world for inspection by the immune system. Hnf4 had not previously been implicated in M cell biology, but gut M cells derive from intestinal stem cells (Gebert et al. 1999), lending biological plausibility to a role for Hnf4 in M cells. Remarkably, examination of *Hnf4g*$^{-/-}$ mice revealed complete loss of gut M cells from Peyer's patches and corresponding defects in the uptake of gut luminal antigens and the generation of intestinal B cell responses. Thus, mimetic cells served as hypothesis-generating reflections of the peripheral self, enabling the discovery of new biology in the gut via study of the thymus.

As another example, tuft cells were recently found to critically depend on two cofactors, Pou2af2 and Pou2af3, that were previously known only as the orphan proteins C11orf53 and Colca2 (Wu et al. 2022). In scRNA-seq of mimetic cells, transcripts encoding these factors are among the top differentially expressed genes defining tuft mTECs, suggesting that the biology of Pou2af2 and Pou2af3 might just as well have been described in the thymus as in any other tissue. Tuft cells, like M cells, are rare cell types that are poorly modeled in vitro and have significant technical challenges associated with their isolation. The study of mimetic cell equivalents may distill key conserved principles of the cell type in question while sidestepping many of the associated technical challenges.

Accordingly, we propose that many more peripheral insights await from the study of mimetic cells. New TF programs may be the most straightforward homologies to discover, given the central role of TFs in mimetic cell biology. But beyond TFs, mimetic cells also conserve key extrinsic signals and intrinsic signaling pathways from the periphery, such as RANK signaling through NF-κB to generate microfold mTECs (Givony et al. 2023, Knoop et al. 2009). Finally, mimetic cells express key functional molecules and often adopt morphological and functional aspects of their peripheral analogs, permitting the study of these facets of peripheral biology as well. The power of mimetic cells to reveal peripheral biology may be particularly pronounced in the human context where difficulties in tissue procurement compound the difficulties of studying rare cell types. In such situations, thymic tissue, which is often resected and otherwise discarded during cardiac surgery, may yield insights via mimetic cells that are otherwise unobtainable.

DISEASE RELEVANCE AND THERAPEUTIC POTENTIAL OF MIMETIC CELLS

Beyond human health, mimetic cells may also yield insights into human disease. Though largely speculative at the moment, defects in mimetic cells may causally underlie some human diseases, and manipulation of mimetic cells may hold therapeutic value.

Human Aire deficiency serves as a useful model of how defective mimetic cells may impact immunological tolerance. Humans with *AIRE* mutations develop the monogenic autoimmune syndrome APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy), characterized by frequent autoimmune hypoparathyroidism, adrenal insufficiency, and chronic mucocutaneous candidiasis as well as a wide range of variably penetrant features, such as autoimmune diabetes, gastritis, and hypogonadism (for a review, see Ferre et al. 2016). A compelling model for mimetic cells in immunological tolerance links each mimetic cell representation in the thymus to tolerance towards its corresponding peripheral counterpart. This concept is borne out by experiments in mice showing that antigen- and tissue-specific autoimmunity develops in the absence of mimetic cell types and their antigens (Givony et al. 2023, Michelson et al. 2022b, Miller et al. 2018, Tao et al. 2023). More work is required to understand the significance of these results for specific human autoimmune diseases.

Operating under this model, several opportunities present themselves for therapeutic intervention. The most straightforward conceptually would involve augmenting or diminishing the abundance of particular mimetic cell types to enhance or diminish tolerance to a particular peripheral correlate. For example, in gut-directed autoimmunity, one could envision increasing the abundance of entero-hepato mTECs in the thymus to promote gut-directed tolerance. Conversely, in gastrointestinal or hepatic cancers, one might diminish the abundance of entero-hepato mTECs to strengthen the antitumor immune response in a directed fashion. Such aims might be accomplished via small molecules or biologics that act on signaling pathways or effectors important for mimetic cells, via gene therapies that edit lineage-defining TFs and other key regulators of mimetic cells, or via cellular therapies that directly transfer mimetic cells of interest. More general proofs of concept have already been established for these ideas by manipulating the RANK-RANKL pathway in mTECs and combining central tolerance defects with an immune checkpoint blockade to augment antitumor immune responses (Benitez et al. 2020, Khan et al. 2014, Policheni et al. 2022, Proekt et al. 2016). A better understanding of how mechanistically mimetic cells produce T cell tolerance—especially whether they produce dominant tolerance in the form of Tregs or recessive tolerance in the form of clonal deletion—will be important in determining the utility of mimetic cells in various tolerizing applications.

Another fascinating potential application for mimetic cells is in regenerative medicine. Remarkable and unexpected work predating the conceptualization of mimetic cells showed that mTECs can be coaxed to grow into functioning components of skin grafts (Bonfanti et al. 2010). Considered in light of our present knowledge of mimetic cells, this result may plausibly reflect the engraftment of keratinocyte mimetic cells into a bona fide skin environment. Building from this result, we suggest that diverse mimetic cells could serve as sources of donor cells in a variety of contexts, such as skin grafts for burn victims, cardiac and skeletal muscle in cardiomyopathies and muscular dystrophies, or neuronal regeneration in Parkinson's disease. Though a substantial amount of work would be required to realize this vision, mimetic cells may have unique potential to serve as sources of autologous donor tissue in a variety of diseases.

CONCLUSION

The already fascinating phenomenon of PTA expression for self-tolerance has been made even more so by the recent (re)discovery of thymic mimetic cells. These shape-shifters use lineage-defining TFs to mirror cell types from throughout the organism and enforce self-tolerance in maturing T cells. Beyond their implications for immunology, mimetic cells also offer fascinating insights into molecular, cellular, developmental, and evolutionary biology writ large. The cooptation of diverse TFs by mimetic cells represents an unusual opportunity to study the pure activities

of TFs, dissociated from their native tissue contexts but nonetheless acting in vivo. Comparison of these results with those of in vitro systems has already helped clarify core principles of these factors. Studies of the *cis*-regulatory landscape of mimetic cells may also yield insights into how the activities of TFs are facilitated or restrained in different chromatin contexts, a process that the tolerogenic factor Aire may influence not just in TECs but also in ESCs and eTACs. Beyond gene regulation, mimetic cells also conserve other key features of their peripheral counterparts such as external developmental cues and signaling pathways. Studies of mimetic cell differentiation and function may offer corresponding insights into the development of peripheral tissues, as has already proved the case with microfold mTECs and gut M cells. Finally, a deeper understanding and integration of all these facets of mimetic cells promise new therapeutic approaches to autoimmune disease, cancer, and tissue regeneration.

SUMMARY POINTS

1. Mimetic cells are thymic epithelial cells that mimic the chromatin, transcriptional, and phenotypic states of extrathymic cell types.
2. Lineage-defining transcription factors (TFs) drive cellular mimicry in mimetic cells.
3. Mimetic cell differentiation may be facilitated by the intrinsic action of lineage-defining TFs, a permissive chromatin landscape, Aire, and/or extrinsic signaling cues.
4. Medullary thymic epithelial cells (mTECs), embryonic stem cells (ESCs), and extrathymic Aire-expressing cells (eTACs) offer three unique perspectives on Aire biology.
5. The study of mimetic cells can reveal the biology of corresponding peripheral cell types.
6. Harnessing mimetic csells may be of therapeutic value in autoimmunity, cancer, or regenerative medicine.

FUTURE ISSUES

1. What is the comprehensive composition of mimetic cells in mice and humans?
2. What lineage-defining TFs control each mimetic cell subtype?
3. What factors are essential in licensing mTECs to produce mimetic cells?
4. Why are some peripheral cell types not represented as thymic mimetic cells?
5. What are the conserved and differential functions of Aire in mTECs, ESCs, and eTACs?
6. What peripheral biology might be revealed by the study of specific mimetic cells?
7. How can mimetic cells be harnessed for medical benefit?

DISCLOSURE STATEMENT

D.M. is a cofounder and member of the Scientific Advisory Board of Zag Inc.

AUTHOR CONTRIBUTIONS

D.A.M. and D.M. conceptualized, wrote, and edited this article.

ACKNOWLEDGMENTS

We thank C. Laplace for figure production and L. Kozinn for help preparing the manuscript. This work was funded by National Institutes of Health grants DK092541 and AI088204 and the JPB Foundation (to D.M.) and GM144273 (for D.A.M.).

LITERATURE CITED

Aaltonen J, Björses P, Perheentupa J, Horelli-Kuitunen N, Palotie A, et al. 1997. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat. Genet.* 17:399–403

Abramson J, Dobes J, Lyu M, Sonnenberg GF. 2024. The emerging family of RORγt^{+} antigen-presenting cells. *Nat. Rev. Immunol.* 24:64–77

Abramson J, Giraud M, Benoist C, Mathis D. 2010. Aire's partners in the molecular control of immunological tolerance. *Cell* 140:123–35

Akagbosu B, Tayyebi Z, Shibu G, Paucar Iza YA, Deep D, et al. 2022. Novel antigen presenting cell imparts T_{reg}-dependent tolerance to gut microbiota. *Nature* 610:752–60

Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, et al. 2002. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 298:1395–401

Antonia SJ, Geiger T, Miller J, Flavell RA. 1995. Mechanisms of immune tolerance induction through the thymic expression of a peripheral tissue-specific protein. *Int. Immunol.* 7:715–25

Ashby KM, Hogquist KA. 2024. A guide to thymic selection of T cells. *Nat. Rev. Immunol.* 24:103–17. Author correction. 2023. *Nat. Rev. Immunol.* 23:697

Bansal K, Michelson DA, Ramirez RN, Viny AD, Levine RL, et al. 2021. Aire regulates chromatin looping by evicting CTCF from domain boundaries and favoring accumulation of cohesin on superenhancers. *PNAS* 118:e2110991118

Bansal K, Yoshida H, Benoist C, Mathis D. 2017. The transcriptional regulator Aire binds to and activates super-enhancers. *Nat. Immunol.* 18:263–73

Baran-Gale J, Morgan MD, Maio S, Dhalla F, Calvo-Asensio I, et al. 2020. Ageing compromises mouse thymus function and remodels epithelial cell differentiation. *eLife* 9:e56221

Bautista JL, Cramer NT, Miller CN, Chavez J, Berrios DI, et al. 2021. Single-cell transcriptional profiling of human thymic stroma uncovers novel cellular heterogeneity in the thymic medulla. *Nat. Commun.* 12:1096

Benitez AA, Khalil-Aguero S, Nandakumar A, Gupta NT, Zhang W, et al. 2020. Absence of central tolerance in Aire-deficient mice synergizes with immune-checkpoint inhibition to enhance antitumor responses. *Commun. Biol.* 3:355

Bleul CC, Corbeaux T, Reuter A, Fisch P, Monting JS, et al. 2006. Formation of a functional thymus initiated by a postnatal epithelial progenitor cell. *Nature* 441:992–96

Bonfanti P, Claudinot S, Amici AW, Farley A, Blackburn CC, et al. 2010. Microenvironmental reprogramming of thymic epithelial cells to skin multipotent stem cells. *Nature* 466:978–82

Bornstein C, Nevo S, Giladi A, Kadouri N, Pouzolles M, et al. 2018. Single-cell mapping of the thymic stroma identifies IL-25-producing tuft epithelial cells. *Nature* 559:622–26

Brennecke P, Reyes A, Pinto S, Rattay K, Nguyen M, et al. 2015. Single-cell transcriptome analysis reveals coordinated ectopic gene-expression patterns in medullary thymic epithelial cells. *Nat. Immunol.* 16:933–41

Brown CC, Rudensky AY. 2023. Spatiotemporal regulation of peripheral T cell tolerance. *Science* 380:472–78

Chen L, Toke NH, Luo S, Vasoya RP, Fullem RL, et al. 2019. A reinforcing HNF4–SMAD4 feed-forward module stabilizes enterocyte identity. *Nat. Genet.* 51:777–85

Danso-Abeam D, Staats KA, Franckaert D, Van Den Bosch L, Liston A, et al. 2013. Aire mediates thymic expression and tolerance of pancreatic antigens via an unconventional transcriptional mechanism. *Eur. J. Immunol.* 43:75–84

Derbinski J, Pinto S, Rosch S, Hexel K, Kyewski B. 2008. Promiscuous gene expression patterns in single medullary thymic epithelial cells argue for a stochastic mechanism. *PNAS* 105:657–62

Devoss J, Hou Y, Johannes K, Lu W, Liou GI, et al. 2006. Spontaneous autoimmunity prevented by thymic expression of a single self-antigen. *J. Exp. Med.* 203:2727–35

Dhalla F, Baran-Gale J, Maio S, Chappell L, Hollander GA, et al. 2020. Biologically indeterminate yet ordered promiscuous gene expression in single medullary thymic epithelial cells. *EMBO J.* 39:e101828

Dobes J, Ben-Nun O, Binyamin A, Stoler-Barak L, Oftedal BE, et al. 2022. Extrathymic expression of Aire controls the induction of effective T_H17 cell-mediated immune response to *Candida albicans*. *Nat. Immunol.* 23:1098–108

Farr AG, Dooley JL, Erickson M. 2002. Organization of thymic medullary epithelial heterogeneity: implications for mechanisms of epithelial differentiation. *Immunol. Rev.* 189:20–27

Farr AG, Rudensky A. 1998. Medullary thymic epithelium: a mosaic of epithelial "self"? *J. Exp. Med.* 188:1–4

Ferre EM, Rose SR, Rosenzweig SD, Burbelo PD, Romito KR, et al. 2016. Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *JCI Insight* 1:e88782

Gardner JM, DeVoss JJ, Friedman RS, Wong DJ, Tan YX, et al. 2008. Deletional tolerance mediated by extrathymic Aire-expressing cells. *Science* 321:843–47

Gardner JM, Liston A. 2022. RORγt-lineage APCs: the Aire apparent. *Sci. Immunol.* 7:eade9240

Gardner JM, Metzger TC, McMahon EJ, Au-Yeung BB, Krawisz AK, et al. 2013. Extrathymic *Aire*-expressing cells are a distinct bone marrow-derived population that induce functional inactivation of $CD4^+$ T cells. *Immunity* 39:560–72

Gavanescu I, Kessler B, Ploegh H, Benoist C, Mathis D. 2007. Loss of Aire-dependent thymic expression of a peripheral tissue antigen renders it a target of autoimmunity. *PNAS* 104:4583–87

Gebert A, Fassbender S, Werner K, Weissferdt A. 1999. The development of M cells in Peyer's patches is restricted to specialized dome-associated crypts. *Am. J. Pathol.* 154:1573–82

Gillis-Buck E, Miller H, Sirota M, Sanders SJ, Ntranos V, et al. 2021. Extrathymic *Aire*-expressing cells support maternal-fetal tolerance. *Sci. Immunol.* 6:eabf1968

Giraud M, Jmari N, Du L, Carallis F, Nieland TJ, et al. 2014. An RNAi screen for Aire cofactors reveals a role for Hnrnpl in polymerase release and Aire-activated ectopic transcription. *PNAS* 111:1491–96

Giraud M, Yoshida H, Abramson J, Rahl PB, Young RA, et al. 2012. Aire unleashes stalled RNA polymerase to induce ectopic gene expression in thymic epithelial cells. *PNAS* 109:535–40

Givony T, Leshkowitz D, Del Castillo D, Nevo S, Kadouri N, et al. 2023. Thymic mimetic cells function beyond self-tolerance. *Nature* 622:164–72

Gu B, Lambert JP, Cockburn K, Gingras AC, Rossant J. 2017. AIRE is a critical spindle-associated protein in embryonic stem cells. *eLife* 6:e28131

Gu B, Zhang J, Chen Q, Tao B, Wang W, et al. 2010. Aire regulates the expression of differentiation-associated genes and self-renewal of embryonic stem cells. *Biochem. Biophys. Res. Commun.* 394:418–23

Guha M, Saare M, Maslovskaja J, Kisand K, Liiv I, et al. 2017. DNA breaks and chromatin structural changes enhance the transcription of autoimmune regulator target genes. *J. Biol. Chem.* 292:6542–54

Gunther T, Chen ZF, Kim J, Priemel M, Rueger JM, et al. 2000. Genetic ablation of parathyroid glands reveals another source of parathyroid hormone. *Nature* 406:199–203

Hassall AH. 1846. *The Microscopic Anatomy of the Human Body: In Health and Disease*. London: S. Highley

Herzig Y, Nevo S, Bornstein C, Brezis MR, Ben-Hur S, et al. 2017. Transcriptional programs that control expression of the autoimmune regulator gene *Aire*. *Nat. Immunol.* 18:161–72

Jolicoeur C, Hanahan D, Smith KM. 1994. T-cell tolerance toward a transgenic β-cell antigen and transcription of endogenous pancreatic genes in thymus. *PNAS* 91:6707–11

Joung J, Ma S, Tay T, Geiger-Schuller KR, Kirchgatterer PC, et al. 2023. A transcription factor atlas of directed differentiation. *Cell* 186:209–29

Kaiser C, Bradu A, Gamble N, Caldwell JA, Koh AS. 2022. AIRE in context: leveraging chromatin plasticity to trigger ectopic gene expression. *Immunol. Rev.* 305:59–76

Kedmi R, Najar TA, Mesa KR, Grayson A, Kroehling L, et al. 2022. A $ROR\gamma t^+$ cell instructs gut microbiota-specific T_{reg} cell differentiation. *Nature* 610:737–43

Khan IS, Mouchess ML, Zhu ML, Conley B, Fasano KJ, et al. 2014. Enhancement of an anti-tumor immune response by transient blockade of central T cell tolerance. *J. Exp. Med.* 211:761–68

Kirchner T, Tzartos S, Hoppe F, Schalke B, Wekerle H, et al. 1988. Pathogenesis of myasthenia gravis. Acetylcholine receptor-related antigenic determinants in tumor-free thymuses and thymic epithelial tumors. *Am. J. Pathol.* 130:268–80

Klein L, Kyewski B, Allen PM, Hogquist KA. 2014. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat. Rev. Immunol.* 14:377–91

Knoop KA, Kumar N, Butler BR, Sakthivel SK, Taylor RT, et al. 2009. RANKL is necessary and sufficient to initiate development of antigen-sampling M cells in the intestinal epithelium. *J. Immunol.* 183:5738–47

Koh AS, Miller EL, Buenrostro JD, Moskowitz DM, Wang J, et al. 2018. Rapid chromatin repression by Aire provides precise control of immune tolerance. *Nat. Immunol.* 19:162–72

Kuroda N, Mitani T, Takeda N, Ishimaru N, Arakaki R, et al. 2005. Development of autoimmunity against transcriptionally unrepressed target antigen in the thymus of Aire-deficient mice. *J. Immunol.* 174:1862–70

Liston A, Lesage S, Wilson J, Peltonen L, Goodnow CC. 2003. Aire regulates negative selection of organ-specific T cells. *Nat. Immunol.* 4:350–54

Liu Z, Farley A, Chen L, Kirby BJ, Kovacs CS, et al. 2010. Thymus-associated parathyroid hormone has two cellular origins with distinct endocrine and immunological functions. *PLOS Genet.* 6:e1001251

Lyu M, Suzuki H, Kang L, Gaspal F, Zhou W, et al. 2022. ILC3s select microbiota-specific regulatory T cells to establish tolerance in the gut. *Nature* 610:744–51

Mayer CE, Zuklys S, Zhanybekova S, Ohigashi I, Teh HY, et al. 2016. Dynamic spatio-temporal contribution of single β5t+ cortical epithelial precursors to the thymus medulla. *Eur. J. Immunol.* 46:846–56

Mayer S. 1888. Zur Lehre von der Schilddrüse und Thymus bei den Amphibien. *Anat. Anz.* 3:97–103

Medawar PB. 1963. Discussion: Role of the thymus in the origin of immunological competence. In *The Immunologically Competent Cell: Its Nature and Origin*, ed. GEW Wolstenholme, J Knight, p. 70. Boston: Little Brown

Meredith M, Zemmour D, Mathis D, Benoist C. 2015. Aire controls gene expression in the thymic epithelium with ordered stochasticity. *Nat. Immunol.* 16:942–49

Metzger TC, Khan IS, Gardner JM, Mouchess ML, Johannes KP, et al. 2013. Lineage tracing and cell ablation identify a post-Aire-expressing thymic epithelial cell population. *Cell Rep.* 5:166–79

Michelson DA, Benoist C, Mathis D. 2022a. CTLA-4 on thymic epithelial cells complements Aire for T cell central tolerance. *PNAS* 119:e2215474119

Michelson DA, Hase K, Kaisho T, Benoist C, Mathis D. 2022b. Thymic epithelial cells co-opt lineage-defining transcription factors to eliminate autoreactive T cells. *Cell* 185:2542–58

Michelson DA, Mathis D. 2022. Thymic mimetic cells: tolerogenic masqueraders. *Trends Immunol.* 43:782–91

Michelson DA, Zuo C, Verzi M, Benoist C, Mathis D. 2023. Hnf4 activates mimetic-cell enhancers to recapitulate gut and liver development within the thymus. *J. Exp. Med.* 220:e20230461

Miller CN, Proekt I, von Moltke J, Wells KL, Rajpurkar AR, et al. 2018. Thymic tuft cells promote an IL-4-enriched medulla and shape thymocyte development. *Nature* 559:627–31

Morimoto J, Matsumoto M, Miyazawa R, Yoshida H, Tsuneyama K, et al. 2022. Aire suppresses CTLA-4 expression from the thymic stroma to control autoimmunity. *Cell Rep.* 38:110384

Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, et al. 1997. Positional cloning of the APECED gene. *Nat. Genet.* 17:393–98

Nishikawa Y, Hirota F, Yano M, Kitajima H, Miyazaki J, et al. 2010. Biphasic Aire expression in early embryos and in medullary thymic epithelial cells before end-stage terminal differentiation. *J. Exp. Med.* 207:963–71

Nishikawa Y, Nishijima H, Matsumoto M, Morimoto J, Hirota F, et al. 2014. Temporal lineage tracing of Aire-expressing cells reveals a requirement for Aire in their maturation program. *J. Immunol.* 192:2585–92

Nusser A, Sagar, Swann JB, Krauth B, Diekhoff D, et al. 2022. Developmental dynamics of two bipotent thymic epithelial progenitor types. *Nature* 606:165–71

Panneck AR, Rafiq A, Schutz B, Soultanova A, Deckmann K, et al. 2014. Cholinergic epithelial cell with chemosensory traits in murine thymic medulla. *Cell Tissue Res.* 358:737–48

Park JE, Botting RA, Dominguez CC, Popescu DM, Lavaert M, et al. 2020. A cell atlas of human thymic development defines T cell repertoire formation. *Science* 367:eaay3224

Policheni AN, Teh CE, Robbins A, Tuzlak S, Strasser A, et al. 2022. PD-1 cooperates with AIRE-mediated tolerance to prevent lethal autoimmune disease. *PNAS* 119:e2120149119

Proekt I, Miller CN, Jeanne M, Fasano KJ, Moon JJ, et al. 2016. LYN- and AIRE-mediated tolerance checkpoint defects synergize to trigger organ-specific autoimmunity. *J. Clin. Investig.* 126:3758–71

Remak R. 1855. *Untersuchungen über die Entwickelung der Wirbelthiere*. London: G. Reimer

Rodewald HR. 2008. Thymus organogenesis. *Annu. Rev. Immunol.* 26:355–88

Rodewald HR, Paul S, Haller C, Bluethmann H, Blum C. 2001. Thymus medulla consisting of epithelial islets each derived from a single progenitor. *Nature* 414:763–68

Rossi SW, Jenkinson WE, Anderson G, Jenkinson EJ. 2006. Clonal analysis reveals a common progenitor for thymic cortical and medullary epithelium. *Nature* 441:988–91

Rossi SW, Kim MY, Leibbrandt A, Parnell SM, Jenkinson WE, et al. 2007. RANK signals from $CD4^{+}3^{-}$ inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. *J. Exp. Med.* 204:1267–72

Rubin SA, Baron CS, Pessoa RC, Duran M, Corbin AF, et al. 2022. Single-cell analyses reveal early thymic progenitors and pre-B cells in zebrafish. *J. Exp. Med.* 219:e20220038

Sansom SN, Shikama-Dorn N, Zhanybekova S, Nusspaumer G, Macaulay IC, et al. 2014. Population and single-cell genomics reveal the *Aire* dependency, relief from Polycomb silencing, and distribution of self-antigen expression in thymic epithelia. *Genome Res.* 24:1918–31

Takaba H, Morishita Y, Tomofuji Y, Danks L, Nitta T, et al. 2015. Fezf2 orchestrates a thymic program of self-antigen expression for immune tolerance. *Cell* 163:975–87

Tao W, Ye Z, Wei Y, Wang J, Yang W, et al. 2023. Insm1 regulates mTEC development and immune tolerance. *Cell. Mol. Immunol.* 20:1472–86

Tomofuji Y, Takaba H, Suzuki HI, Benlaribi R, Martinez CDP, et al. 2020. Chd4 choreographs self-antigen expression for central immune tolerance. *Nat. Immunol.* 21:892–901

Villasenor J, Besse W, Benoist C, Mathis D. 2008. Ectopic expression of peripheral-tissue antigens in the thymic epithelium: probabilistic, monoallelic, misinitiated. *PNAS* 105:15854–59

Wang J, Lareau CA, Bautista JL, Gupta AR, Sandor K, et al. 2021. Single-cell multiomics defines tolerogenic extrathymic Aire-expressing populations with unique homology to thymic epithelium. *Sci. Immunol.* 6:eabl5053

Waterfield M, Khan IS, Cortez JT, Fan U, Metzger T, et al. 2014. The transcriptional regulator Aire coopts the repressive ATF7ip-MBD1 complex for the induction of immunotolerance. *Nat. Immunol.* 15:258–65

Watney H. 1882. The minute anatomy of the thymus. *Philos. Trans. R. Soc.* 173:1063–123

Wells KL, Miller CN, Gschwind AR, Wei W, Phipps JD, et al. 2020. Combined transient ablation and single-cell RNA-sequencing reveals the development of medullary thymic epithelial cells. *eLife* 9:e60188

Wu XS, He XY, Ipsaro JJ, Huang YH, Preall JB, et al. 2022. OCA-T1 and OCA-T2 are coactivators of POU2F3 in the tuft cell lineage. *Nature* 607:169–75

Yamano T, Dobes J, Voboril M, Steinert M, Brabec T, et al. 2019. Aire-expressing ILC3-like cells in the lymph node display potent APC features. *J. Exp. Med.* 216:1027–37

Yoshida H, Bansal K, Schaefer U, Chapman T, Rioja I, et al. 2015. Brd4 bridges the transcriptional regulators, Aire and P-TEFb, to promote elongation of peripheral-tissue antigen transcripts in thymic stromal cells. *PNAS* 112:E4448–57