

Thymic epithelial cell heterogeneity: TEC by TEC

Noam Kadouri, Shir Nevo, Yael Goldfarb and Jakub Abramson *

Abstract | The generation of a functional T cell repertoire in the thymus is mainly orchestrated by thymic epithelial cells (TECs), which provide developing T cells with cues for their navigation, proliferation, differentiation and survival. The TEC compartment has been segregated historically into two major populations of medullary TECs and cortical TECs, which differ in their anatomical localization, molecular characteristics and functional roles. However, recent studies have shown that TECs are highly heterogeneous and comprise multiple subpopulations with distinct molecular and functional characteristics, including tuft cell-like or corneocyte-like phenotypes. Here, we review the most recent advances in our understanding of TEC heterogeneity from a molecular, functional and developmental perspective. In particular, we highlight the key insights that were recently provided by single-cell genomic technologies and in vivo fate mapping and discuss them in the context of previously published data.

Thymic epithelial cells

(TECs). Specialized stromal cells found in the thymus that have the ability to present antigens on MHC class I and class II molecules to developing T cells (thymocytes). Their main known functions include the induction of T cell lineage commitment, positive selection of functional T cell clones and negative selection of self-reactive T cell clones.

Positive selection

A crucial checkpoint in $\alpha\beta$ T cell development, exclusively facilitated by cortical thymic epithelial cells, that ensures only functionally competent T cell clones capable of recognizing peptide–MHC complexes with adequately high affinity continue in the developmental process. T cell clones that do not recognize peptide–MHC complexes with sufficient affinity die by neglect.

Department of Immunology,
Weizmann Institute of
Science, Rehovot, Israel.

*e-mail: jakub.abramson@weizmann.ac.il

<https://doi.org/10.1038/s41577-019-0238-0>

The thymus is a specialized primary lymphoid organ whose main function is the production of immunologically competent T cells that can recognize and eliminate foreign antigens but that tolerate the body's own components¹. T cell 'education' in the thymus is mainly orchestrated by thymic epithelial cells (TECs), which provide developing T cells with cues for their navigation, proliferation, differentiation and survival (BOX 1).

The TEC compartment has been divided historically into two major subsets, which differ in their anatomical localization and functional roles. Specifically, whereas the early checkpoints of the T cell developmental programme (T cell lineage commitment and positive selection) are orchestrated by cortical thymic epithelial cells (cTECs), later steps of T cell development, including negative selection of self-reactive thymocytes or their diversion into the FOXP3⁺CD25⁺ regulatory T (T_{reg}) cell lineage (agonist selection), are primarily mediated by medullary thymic epithelial cells (mTECs)^{2,3}.

In the past several years, however, it has become evident that cTECs and mTECs are not homogeneous compartments but rather are characterized by a high degree of internal heterogeneity (FIG. 1a). This increasing appreciation of TEC complexity, previously reviewed in REF.⁴, is accompanied by growing confusion regarding the molecular and functional characterization of the individual TEC subsets. This may stem from the fact that the characterization and/or isolation of these subsets has been achieved using only a small number of surface markers, hence obscuring the composite picture of the TEC compartment.

Here, we review the most recent advances in our understanding of TEC heterogeneity from a molecular, functional and developmental perspective that have been provided by single-cell genomic technologies and in vivo fate mapping and discuss them in the context of previously published data. Specifically, we discuss thymic epithelial progenitor cells (TEPCs) and highlight some of the key open questions and controversies regarding their molecular characteristics and progenitor properties at different stages of thymic development. Furthermore, we review in detail our current understanding of cTEC and mTEC heterogeneity and development, with a particular focus on the diverse cell subsets that were recently found to compose the autoimmune regulator (AIRE)-negative mTEC compartment, including the mTEC I subset⁵, CC-chemokine ligand 21 (CCL21)-expressing mTECs^{6,7}, podoplanin-expressing (PDPN⁺) junctional thymic epithelial cells (jTECs)^{8,9}, corneocyte-like keratin type I cytoskeletal 10-positive (KRT10⁺) mTECs (also known as post-AIRE mTECs or mTEC III)^{5,10–12} and the newly identified DCLK1⁺ thymic tuft cells (also known as mTEC IV)^{5,13–15}. We hope that this Review not only provides the most up-to-date and comprehensive snapshot of the TEC atlas but also helps in integrating this information with previous knowledge and clarifying some of the outstanding questions from the past.

Thymic epithelial progenitor cells

Identification of the putative TEPCs has been one of the major challenges in the field. Despite marked progress in this direction in the past two decades, and convincing evidence for the existence of bipotent TEPCs that can

Box 1 | T cell education

The thymus is the only tissue in the body that can support the development of lymphoid progenitors into mature and immunologically competent naive T cells. Specifically, early thymic progenitors are recruited to the thymus where, upon encountering Notch ligands (in particular, delta-like ligand 4 (DLL4)) provided by cortical thymic epithelial cells (cTECs), they irreversibly commit to the T cell lineage. Moreover, cTECs have a crucial role in controlling the subsequent steps of the T cell developmental programme (until the CD4⁺CD8⁺ double-positive thymocyte stage), including clonal expansion and positive selection of T cell clones that express functional $\alpha\beta$ T cell receptors (TCRs). Specifically, the highly broad and diverse repertoire of TCRs that is generated by the semi-random process of VDJ recombination is tested for its ability to bind peptide–MHC (pMHC) complexes that are exclusively presented by cTECs. To this end, cTECs express a unique proteasome (known as the thymoproteasome) and lysosomal proteases that enable distinctive cleavage and subsequent presentation of the self-antigen repertoire. Interactions between a presented antigenic peptide and its corresponding TCR result in survival of the T cell clone and its progression to either the CD4⁺ or CD8⁺ single-positive subset, whereas failure of a thymocyte to bind such antigens leads to its death by neglect. The surviving single-positive thymocytes migrate into the thymic medulla, where potentially dangerous clones expressing self-reactive TCRs are pruned from the repertoire through a process known as negative selection. This step is mainly mediated by dendritic cells and medullary thymic epithelial cells (mTECs). Importantly, to effectively screen for as many self-reactive thymocyte clones as possible, mTECs are equipped with a unique capacity to express almost 90% of the coding genome, including tissue-restricted antigens. Peptides derived from these self-antigens are presented on MHC molecules on the surface of mTECs or are transferred to neighbouring dendritic cells for cross-presentation, which further potentiates the selection process. High-affinity interaction between a TCR and self-pMHC most commonly results in clonal deletion, whereas interactions of low to intermediate affinity typically divert a given clone into the FOXP3⁺CD4⁺ regulatory T cell lineage, a process known as agonist selection. Functional defects in either cTECs and/or mTECs are associated with diverse immunopathologies, ranging from severe immunodeficiency to autoimmunity.

Negative selection

A selection process mediated by thymus-resident antigen-presenting cells (for example, medullary thymic epithelial cells, dendritic cells and B cells) that ensures T cell clones that recognize self-peptide–MHC complexes with very high affinity are eliminated from the repertoire. This process occurs mainly in the thymic medulla, although there is some evidence that negative selection can also occur in the cortex.

Agonist selection

A selection process that ensures CD4⁺ T cell clones that recognize self-peptide–MHC class II complexes with medium to high affinity differentiate into CD4⁺CD25⁺FOXP3⁺ regulatory T cells. It has been suggested that this process is mediated by medulla-resident antigen-presenting cells (such as medullary thymic epithelial cells, dendritic cells and B cells).

concurrently give rise to both mTEC and cTEC lineages in the fetal and early neonatal thymus, a substantial degree of controversy remains regarding the exact identity and molecular characteristics of TEPCs, particularly in the adult thymus. Specifically, although several studies have proposed the existence of a bipotent TEPC^{16–19}, others have argued that mTECs and cTECs are maintained by lineage-specific progenitors^{20,21}. Moreover, many of these studies differ markedly in the molecular characterization of such putative TEPCs, each proposing a different set of markers for their identification.

One of the first pieces of evidence to support the existence of bipotent TEPCs in the embryonic thymus comes from experiments showing that a single EPCAM⁺CD45⁻ cell isolated from the thymus at embryonic day 12.5 gives rise to both cTEC and mTEC progeny after transplantation²². Similarly, another study using an *in vivo* fate-mapping approach showed that a single embryonic TEC gives rise to distinct cell islets within the thymus, comprising both cTECs and mTECs (in 76% of the cases) or the individual lineages, which suggests that both bipotent and lineage-restricted TEPCs exist in the embryonic thymus²³ (FIG. 1b). These findings are further supported by two more recent studies^{20,21} that carried out *in vivo* lineage tracing using a fluorescent reporter activated by a doxycycline-inducible Cre recombinase under control of the *Psmb11* promoter. Importantly, although *Psmb11* is a cTEC-specific gene encoding the $\beta 5t$ subunit of the thymoproteasome, it was also previously shown to be active in embryonic bipotent TEPCs²⁴. Using this

elegant fate-mapping system, both studies^{20,21} showed that the *Psmb11*-expressing TEPCs are still present in the neonatal thymus but that their progenitor capacity disappears ~2 weeks after birth. Although this supports the conclusion that postnatal $\beta 5t$ -expressing cells are not bipotent progenitors in the adult thymus, it does not necessarily show that there is a lack of bipotency within the $\beta 5t$ ⁻ population, as suggested by REFS^{17,19} (FIG. 1b).

The molecular characterization of the embryonic TEPCs has been marked by several discrepancies between groups. Specifically, in 2002, two independent groups showed that the embryonic thymus contains bipotent TEPCs that are characterized by expression of the surface marker PLET1, which is recognized by the monoclonal antibody MTS24 (REFS^{25,26}). However, a more recent study using larger cell numbers showed that both PLET1⁺ and PLET1⁻ embryonic TECs have a similar capacity to generate functional thymic tissue²⁷. As the phenotype of the transplanted PLET1⁻ cells was not determined in the later study²⁷, it cannot be excluded that PLET1⁻ lineage-restricted TEPCs could arise from PLET1⁺ bipotent TEPCs. Thus, whether PLET1 expression marks bipotent TEPCs remains a point of discussion. Importantly, several subsequent studies have shown that embryonic TEPCs are characterized by a strong ‘cTEC footprint’, including high levels of expression of $\beta 5t$ ²⁴ and CD205 (also known as LY75)²⁸, which are highly specific to the cTEC lineage in the adult thymus⁴. Based on these findings, a ‘serial progression model’ of TEC development has been proposed²⁹, according to which bipotent progenitors commit to the cTEC lineage by default (FIG. 1b), whereas entry into the mTEC lineage requires activation of an mTEC-specific transcriptional programme concomitant with the downregulation of cTEC-specific genes. Importantly, this model is further supported by recent single-cell RNA sequencing (scrRNA-seq) analysis⁵, which showed that most embryonic TECs are characterized by a strong cTEC footprint and relatively limited cell heterogeneity. Interestingly, in spite of their marked similarities to mature cTECs, the embryonic TEPCs have several unique characteristics that distinguish them from adult cTECs, including high levels of expression of cell cycle-related genes, fibronectin 1 (*Fn1*) and pyruvate kinase 2 (*Pkm*), and low levels of expression of genes of the MHC class II pathway⁵ (FIG. 1c). These data therefore collectively suggest that although the bipotent TEPCs in the embryonic thymus may share many molecular characteristics with mature cTECs, they likely differ markedly in their proliferative, metabolic or antigen-presentation properties and, most importantly, in their progenitor capacities. Moreover, the proportion of putative TEPCs among total TECs and/or their progenitor capacity seem to progressively decrease with embryonic age to undetectable levels in the adult thymus, reflecting the gradual emergence of additional TEC subsets corresponding to the cTEC and mTEC lineages^{5,20,21}.

The existence and molecular characterization of bipotent TEPCs in the adult thymus still remain largely controversial. Several independent studies have shown that the adult thymus does not contain cTEC-like bipotent TEPCs^{20,21}, whereas other studies have suggested that

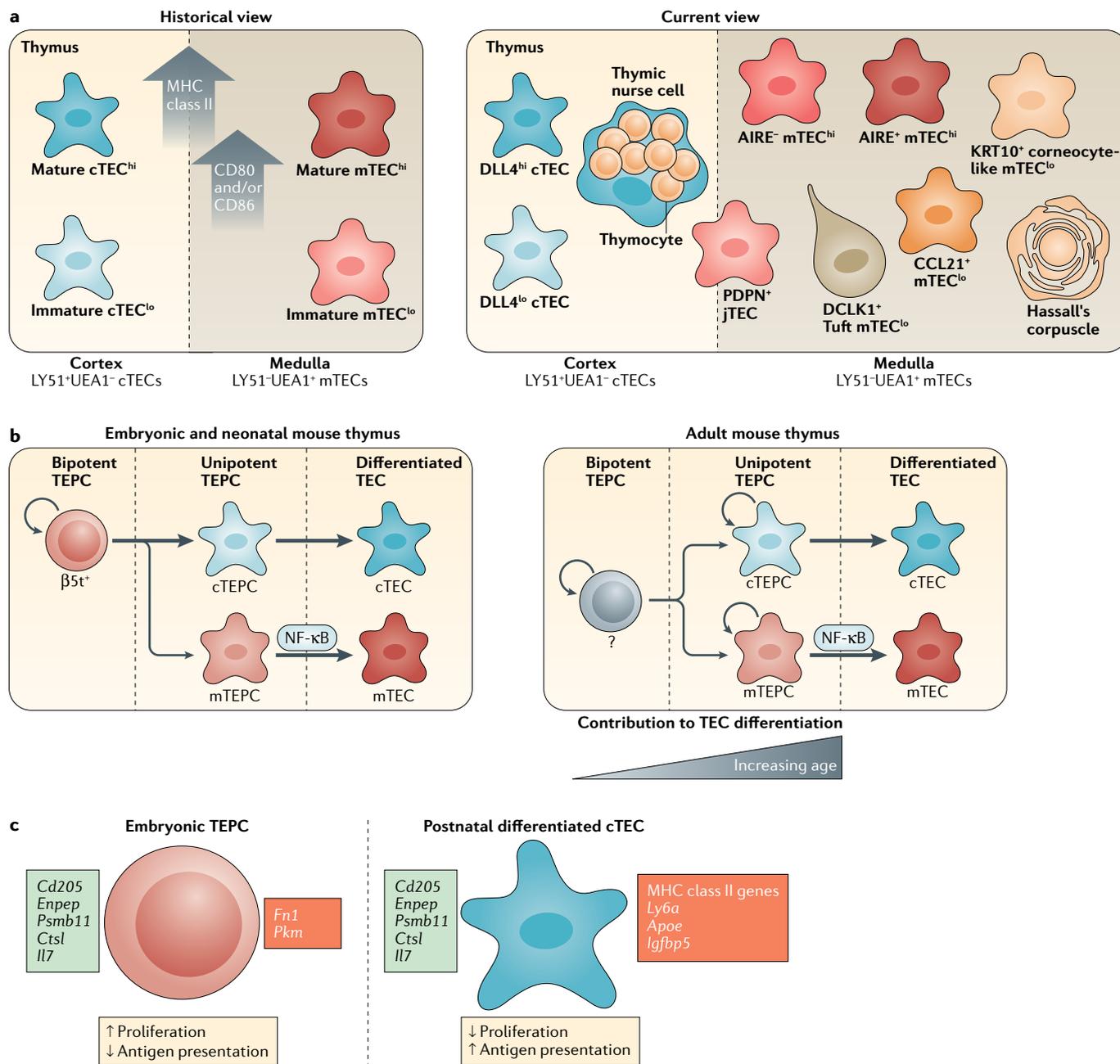


Fig. 1 | Thymic epithelial cell heterogeneity and development. **a** | Historical versus current views of thymic epithelial cell (TEC) heterogeneity. Historically (left panel), medullary thymic epithelial cells (mTECs) and cortical thymic epithelial cells (cTECs) were differentiated using only a small number of surface markers, such as UEA1 (for mTECs), LY51 (for cTECs) or MHC class II and/or CD80 and CD86 (for the segregation of less mature TECs from more mature TEC developmental states). Recent technological advances have been used to show that there is a high level of TEC heterogeneity (right panel), exemplified by the existence of various cTEC and mTEC subpopulations with distinct molecular and functional characteristics. **b** | Current models for TEC development. The development of TECs from a thymic epithelial progenitor cell (TEPC) differs between the embryonic mouse thymus and the adult mouse thymus. In the embryonic thymus (left panel), bipotent $\beta 5t^+$ TEPCs can ultimately give rise to both mTEC and cTEC lineages, a capacity that is maintained during neonatal life. The bipotent TEPCs commit to the cTEC lineage by default (thick arrow), whereas entry into the mTEC lineage (thin arrow) requires activation of an mTEC-specific transcriptional programme, including activation of the

nuclear factor- κ B (NF- κ B) signalling pathway. In the adult thymus (right panel), the existence of a bipotent TEPC remains controversial, with a prevailing assumption that even if such bipotent progenitors exist, the contribution of lineage-restricted TEPCs to the maintenance of both cTECs and mTECs increases with age. **c** | Similarities and differences between embryonic TEPCs and differentiated cTECs. Although embryonic TEPCs have a strong molecular 'footprint' of differentiated cTECs, including high levels of expression of *Psmb11* and *Cd205* (similarly expressed genes are shown in green), they differ from differentiated cTECs in terms of increased proliferation and decreased antigen-presentation capacities, as well as differential expression of various key genes (shown in red). AIRE, autoimmune regulator; *Apoe*, gene encoding apolipoprotein E; CCL21, CC-chemokine ligand 21; cTEPC, cTEC progenitor; *Ctsl*, gene encoding cathepsin L1; DLL4, delta-like ligand 4; *Enpep*, gene encoding LY51; *Fn1*, gene encoding fibronectin 1; *Igfbp5*, gene encoding insulin-like growth factor-binding protein 5; jTEC, junctional TEC; KRT10, keratin type I cytoskeletal 10; mTEPC, mTEC progenitor; PDPN, podoplanin; *Pkm*, gene encoding pyruvate kinase 2; *Psmb11*, gene encoding $\beta 5t$.

bipotent TEPCs do exist in the adult thymus, albeit with different molecular characteristics^{16,17,19}. For example, a rare CD45⁺EPCAM⁺PLET1⁺UEA1⁻LY51⁺MHCII^{hi} TEC subset was proposed to have bipotent capacity when transferred into reaggregate thymic organ cultures (RTOCs)¹⁶. By contrast, another study suggested that bipotent TEPCs are contained within a small subset of CD45⁺EPCAM⁺LY51^{lo}UEA1^{lo}MHCII^{lo}ITGA6^{hi}SCA1^{hi} TECs in RTOC-based experiments¹⁷, and an additional study reported a similar population that is radioresistant and proliferates during thymus regeneration³⁰. Moreover, in vitro analysis of TEC subsets using a colony-forming assay showed that EPCAM⁺LY51^{lo}UEA1^{lo}MHCII^{lo}ITGA6^{hi}SCA1^{hi} TECs have the greatest capacity to form colonies¹⁷ that, upon transplantation under the kidney capsule, gave rise to functionally mature cTEC and mTEC lineages¹⁷. However, the exact molecular characteristics of such adult bipotent TEPCs are still largely unknown. Recent scRNA-seq analysis of the adult TEC compartment showed that *Itga6* and *Sca1*, which were proposed to mark bipotent TEPCs in the adult thymus¹⁷, are highly enriched in at least two distinct cell clusters, one having a cTEC-specific gene signature and the other having an mTEC-specific gene signature⁵. Thus, *ITGA6* and *SCA1* may not suffice for the characterization of bipotent TEPCs, and the identification of additional and more specific markers is needed. Finally, another study has proposed that bipotent TEPCs in the adult thymus form spheric organoids (termed thymospheres), a feature that is characteristic of stem cells in other tissues, and do not express several key hallmarks of TECs, such as EPCAM and FOXP1 (REF.¹⁸). However, these findings were recently challenged by another study showing that the thymosphere-forming cells are mainly mesenchymal cells that can incorporate bystander TECs into the thymosphere³¹. Recent evidence has shown that although bipotent TEPCs may exist in the adult thymus, replenishment of the TEC population after puberty relies heavily upon lineage-restricted TEPCs, as the bipotent TEPCs become quiescent¹⁹. The underlying mechanisms that account for this change in TEPC activity are the result of age-dependent increases in levels of both follistatin (FST) and bone morphogenetic protein 4 (BMP4). FST antagonizes activin A signalling, which is an important factor for TEC differentiation, and BMP4 has been shown to support the maintenance of TEPCs^{19,32}.

In summary, although there is sufficient evidence to support the existence of bipotent TEPCs in the embryonic and neonatal thymus, the existence of such cells in the adult thymus remains controversial and requires further experimental support based on in vivo single-cell fate-mapping experiments. Moreover, future studies should provide detailed molecular characterization of such TEPCs, particularly in the adult thymus.

Cortical thymic epithelial cells

cTECs are equipped with several key molecules that are crucial for the regulation of different checkpoints of the initial T cell developmental programme^{4,33} (FIG. 2a). Specifically, mature cTECs express high levels of two key cytokines, CXC-chemokine ligand 12 (CXCL12) and CC-chemokine ligand 25 (CCL25), which promote

the homing of blood-borne lymphoid progenitor cells into the thymus^{34–37}. Moreover, cTECs express high levels of the Notch ligand delta-like ligand 4 (DLL4), which instructs the recruited lymphoid progenitors to commit to the T cell lineage^{38,39}. Mature cTECs also express various cytokines, such as IL-7 and stem cell factor (SCF), that promote the proliferation and survival of the developing thymocytes⁴⁰. Being the exclusive mediators of positive selection of immunocompetent T cells in the thymus¹, cTECs express specific components of the antigen-processing and presentation machinery, including the thymoproteasome subunit $\beta 5t$ (encoded by *Psmb11*) and the proteases cathepsin L1 (encoded by *Ctsl*) and thymus-specific serine protease (TSSP; encoded by *Prss16*)⁴¹. Recently, and relevant to their role in positive selection, cTECs were also found to express CD83 (REF.⁴²), which regulates the turnover of surface MHC class II molecules and thereby affects the development of CD4⁺ single-positive thymocytes^{43,44}.

The heterogeneity of cTECs is still poorly understood and probably largely underestimated. In general, most cTECs in the postnatal thymus are defined by high levels of expression of the surface markers LY51 (also known as CD249; encoded by *Enpep*), LY75 (encoded by *Cd205*) and MHC class II. However, a small fraction of MHCII^{lo}LY51⁺LY75⁺ cTECs also exists, likely representing an immature subset⁴⁵. cTECs were also found to have highly heterogeneous levels of expression of DLL4, which depend on the developmental stage of the thymus and decrease with age⁴⁶. Whereas almost all embryonic cTECs express high levels of DLL4, a large fraction of cTECs in the postnatal thymus expresses lower levels of DLL4 (REF.⁴⁶). Moreover, the existence of two major cTEC subsets distinguished by differential expression of *Dll4* — as well as additional genes, such as *Cd83*, *Ackr4* (also known as *Ccrl1*), *Ccl25*, *Ly6a* and *Cxcl12* — was recently validated by scRNA-seq analysis⁵ (FIG. 2b), but whether these DLL4^{hi} and DLL4^{lo} cTEC subsets have different functional roles remains to be investigated.

It is also important to stress that the limited cTEC heterogeneity highlighted by scRNA-seq analysis⁵ may be largely underestimated, as such single cell-based analyses may have ‘filtered out’, and thus overlooked, a large fraction of cTECs that are tightly associated with thymocytes. In particular, such complex cTEC structures may include thymic nurse cells (TNCs) or their precursors (FIG. 2b), which are a significant fraction of cTECs in the adult thymus^{47,48}. Although detailed molecular characterization of TNCs is still missing, they were shown to be capable of internalizing between 2 and 200 immature double-positive thymocytes⁴⁹. TNCs therefore resemble a complex ‘organoid’ more than a conventional cell (FIG. 2b). The engulfment of developing thymocytes by TNCs was shown to be crucial for secondary T cell receptor (TCR) α -chain rearrangement⁴⁸. Furthermore, whereas some of the internalized thymocytes proliferate and mature into TCR^{hi}CD69⁺ double-positive T cells, which are then released from the TNC, others undergo apoptosis and are degraded within the TNC⁴⁹.

The developmental heterogeneity of cTECs is also poorly understood. Although the cTEC compartment can fully regenerate following cTEC-specific ablation

Autoimmune regulator

(AIRE). A transcriptional regulator, expressed almost exclusively in mature MHCII^{hi} medullary thymic epithelial cells. It induces the expression of most tissue-restricted antigen genes in the thymus, a step that is necessary for purging of self-reactive T cells and induction of central tolerance.

Thymoproteasome

A specialized form of the proteasome that is found exclusively in cortical thymic epithelial cells and that is essential for the generation of a unique peptide repertoire to support positive selection of T cell clones. The thymoproteasome uniquely incorporates the $\beta 5t$ subunit (encoded by *Psmb11*).

Reaggregate thymic organ cultures

(RTOC). An experimental method that enables the ex vivo generation of three-dimensional thymic organoids from purified fetal thymic epithelial cells and other thymic cell subsets. The resulting organoids can also be used in transplantation studies for a longer period of time.

Thymic nurse cells

(TNCs). Large cortical thymic epithelial structures that internalize developing thymocytes through extensions of the plasma membrane. Thymic nurse cells can internalize up to 200 double-positive thymocytes and have been shown to be crucial for secondary T cell receptor α -chain rearrangement.

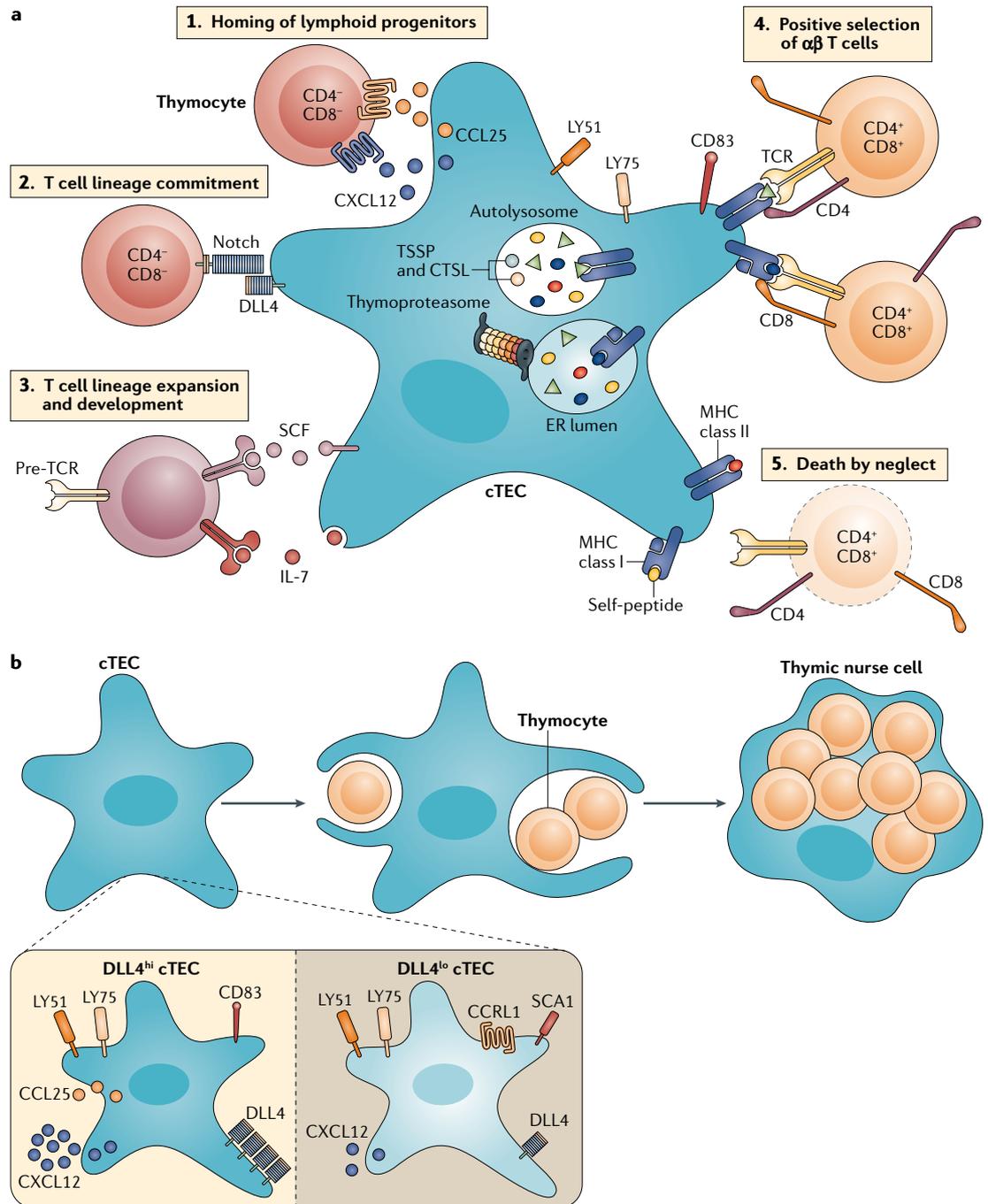


Fig. 2 | Function and diversity of cortical thymic epithelial cells. **a** | Functions of cortical thymic epithelial cells (cTECs). cTECs, which are characterized by surface expression of LY51 and LY75, have important roles in the initial stages of T cell development and selection, including: **(1)** homing of blood-borne lymphoid progenitor cells into the thymus, through the expression of CC-chemokine ligand 25 (CCL25) and CXC-chemokine ligand 12 (CXCL12); **(2)** commitment of the lymphoid progenitors to the T cell lineage, through expression of the Notch ligand delta-like ligand 4 (DLL4); **(3)** expansion of the developing thymocyte clones, through secretion of IL-7 and stem cell factor (SCF; also known as KIT ligand); **(4)** positive selection of thymocytes; and **(5)** death by neglect of thymocytes bearing a T cell receptor (TCR) that does not recognize peptides presented by an MHC complex. Positive selection of thymocytes is supported by cTEC expression of a specific antigen-processing and presentation machinery, including the use of autophagy to obtain self-proteins for processing using the thymoproteasome, which uniquely expresses the $\beta 5t$ subunit, as well as processing by the proteases cathepsin L1 (CTSL) and thymus-specific serine protease (TSSP). In addition, cTECs express CD83, which regulates the turnover of surface MHC class II molecules. **b** | cTEC heterogeneity. In the adult thymus, two major cTEC subsets were recently described by single-cell RNA sequencing analysis, based on the differential expression of DLL4 as well as of additional key genes, including *Ccl25*, *Cxcl12*, *Cd83*, *Ly6a* (which encodes SCA1) and *Ccr11*. The heterogeneity of cTECs is further increased by the existence of thymic nurse cells, which engulf and subsequently 'nurture' developing thymocytes. A single thymic nurse cell can engulf a large number of thymocytes. CCRL1, CC-chemokine receptor-like 1; ER, endoplasmic reticulum.

in adult mice⁵⁰, little is known about the putative cTEC progenitor cells. One such population that has been briefly described is short-term cTEC progenitor cells in the PLET1⁺LY51⁺ fraction in the adult thymus¹⁶. More recently, SCA1^{lo}MHCII^{lo} cTECs were shown to be precursors of mature cTECs, and the contribution of these precursors to cTEC maintenance increases with age¹⁹. Moreover, unlike mTEC development, which is orchestrated by nuclear factor- κ B (NF- κ B) signals (see below)⁵¹, no specific signalling pathway has yet been identified to regulate cTEC development.

mTEC progenitor cells and immature mTECs

The cell precursors that give rise to and/or maintain the mTEC compartment seem to differ greatly between the embryonic thymus and the adult thymus. Specifically, whereas the embryonic mTEC progenitors (mTEPCs) are defined by high levels of expression of the tight junction components claudin 3 (CLDN3) and CLDN4, progenitors that maintain the mTEC compartment in the adult thymus do not express CLDN3 or CLDN4 (REF.⁵²) and express a different set of molecular markers (discussed below).

A small subset of the CLDN3^{hi}CLDN4^{hi} embryonic mTEPCs was shown to have clonogenic capacity, which suggests that it may comprise unipotent mTEC stem cells⁵³. Specifically, these CLDN3^{hi}CLDN4^{hi} mTEC stem cells were found to express stage-specific embryonic antigen 1 (SSEA-1; also known as CD15) and to be present in the embryonic thymus of nude mice and RELB-deficient mice, which suggests that they do not require FOXN1 or NF- κ B signalling for their emergence and subsequent maintenance⁵⁴. The NF- κ B signalling pathway was, however, crucial for the upregulation of expression of receptor activator of NF- κ B (RANK; also known as TNFRSF11A) in these mTEC stem cells, a step that is necessary for subsequent stages of mTEC development^{54,55} (FIG. 1b). In addition to RANK, other members of the tumour necrosis factor receptor superfamily^{55–59}, including CD40 (REFS^{55,60–63}) and lymphotoxin- β receptor (LT β R)^{57,64,65}, were also found to have key roles in controlling different checkpoints of the mTEC developmental programme.

Interestingly, the CLDN3^{hi}CLDN4^{hi} mTECs in the adult thymus do not have any progenitor capacity and are instead restricted to mature AIRE⁺ and terminally differentiated mTECs⁵². Correspondingly, the identification of analogous markers that specifically define mTEC precursors in the postnatal thymus has been more challenging and only partially successful. Several initial studies suggested that the postnatal mTEPCs are contained within the MHCII^{lo}CD80^{lo} mTEC compartment (known as mTEC^{lo} cells), as this population was shown to give rise to mature AIRE⁺MHCII^{hi}CD80^{hi} cells (known as mTEC^{hi} cells)^{27,66}. However, the relatively high abundance and the recently discovered heterogeneity of the mTEC^{lo} compartment suggest that mTEPCs likely constitute only a small fraction of this population^{5,13,61,66–69}. More recently, it was suggested that the putative mTEC^{lo} progenitors in the postnatal thymus are characterized by high levels of expression of the surface protein PDPN and several genes encoding various CC-chemokine receptor 7 (CCR7) ligands, including

Ccl21a, *Ccl21b* and *Ccl21c*⁸. Moreover, the same study showed that these PDPN⁺ mTEC^{lo} cells preferentially localize at the cortico-medullary junction — thus being known as jTECs — and accumulate in mice with mTEC-specific deletion of the NF- κ B-inducing kinase (NIK)⁸. Importantly, the existence of this PDPN⁺ jTEC subset was subsequently supported by data from two independent scRNA-seq studies^{5,9}. Specifically, one study identified a distinct mTEC cluster that was characterized by co-expression of *Pdpr*, *Ccl21a*, *Ccl21b* and *Lgals1*; based on pseudotime analysis, it was suggested that this cluster is the earliest mTEC population in the adult thymus⁹. Another study highlighted a rather large and heterogeneous MHCII^{lo} cell cluster, referred to as the mTEC I subset, that was characterized by high-level and specific expression of genes encoding various CCR7 ligands (such as *Ccl21a* and *Ccl21c*), surface markers (such as *Itgb4*, *Itga6* and *Lifr*), transcription factors (such as *Sox4* and *Ascl1*) and mTEC-specific cytokeratins (such as *Krt5* and *Krt14*)⁵. Importantly, the mTEC I cluster also contained a small, but distinct, subset characterized by co-expression of *Pdpr*, *Sca1* and *Lgals1* (REF.⁵), which suggests that the putative PDPN⁺ mTEPCs constitute only a small fraction of the *Ccl21a*- and/or *Ccl21c*-expressing mTEC I cells (FIG. 3). It is also important to note that the CCR7 ligand-producing (in other words, CCL21⁺) mTEC^{lo} cells were previously suggested to be a lymphotoxin signalling-dependent post-AIRE population⁶, rather than an early mTEC subset with putative progenitor capacity. This notion was supported by the fact that, during mouse ontogeny, the CCL21⁺ mTECs appear only after the emergence of AIRE⁺ cells, and they are significantly under-represented in AIRE-deficient thymi⁶. However, subsequent studies using AIRE reporter mice showed that several key CCR7 ligands (encoded by *Ccl21a* and *Ccl19*) are not expressed in post-AIRE cells but rather in a separate mTEC subset that developmentally precedes the expression of *Aire* in the adult thymus¹². Moreover, in vivo cell fate-mapping analysis using *Csn2*^{Cre}+*Rosa26*^{tdTomato} reporter mice showed that the mTEC I population, which is characterized by specific expression of *Ccl21a* and *Ccl21c*, is the only mTEC subset that is not developmentally derived from mature mTECs, which supports the notion that these cells are at an early stage rather than a late stage of mTEC development. Interestingly, although the mTEC I cells were not derived from mature mTEC^{hi} cells, their cellularity was markedly reduced in AIRE-deficient thymi⁵, which suggests that their survival and/or proliferation are regulated by AIRE⁺ mTECs (FIG. 3). Although the molecular mechanisms underlying this cellular crosstalk remain elusive, they may possibly involve paracrine signalling from AIRE⁺ mTECs (FIG. 3).

Taken together, the currently available experimental evidence suggests that the mTEC I population is likely to be composed of at least two functionally distinct subsets: putative mTEPCs that replenish the mTEC compartment in the adult thymus; and a developmentally mature CCL21-producing population, whose major role is to recruit (and/or retain) positively selected CCR7⁺ thymocytes into the medulla^{6,70} (FIG. 3). Additional in vivo studies will be required to further validate such a

Nude mice

A mouse strain having a naturally occurring loss-of-function mutation in the Forkhead box N1 (*Foxn1*) gene, which encodes a transcription factor that is crucial for the development of hair follicles, mammary glands and thymic epithelial cells. As a result, nude mice develop no hair and have a dysfunctional thymic rudiment, which is unable to support normal T cell development, resulting in severe immunodeficiency.

Csn2^{Cre}+*Rosa26*^{tdTomato} reporter mice

Csn2^{Cre} mice are a transgenic mouse model in which the coding sequence for Cre recombinase is inserted downstream of the *Csn2* gene promoter. *Rosa26*^{tdTomato} mice are a transgenic mouse model in which the *tdTomato* reporter gene, together with a stop cassette flanked by *loxP* sites, is inserted into the *Rosa26* locus. As *Csn2* is specifically expressed in most MHCII^{hi}CD80^{hi} (mTEC^{hi}) cells, the *Csn2*^{Cre}+*Rosa26*^{tdTomato} reporter mice enable lineage tracing of mTEC^{hi}-derived cells.

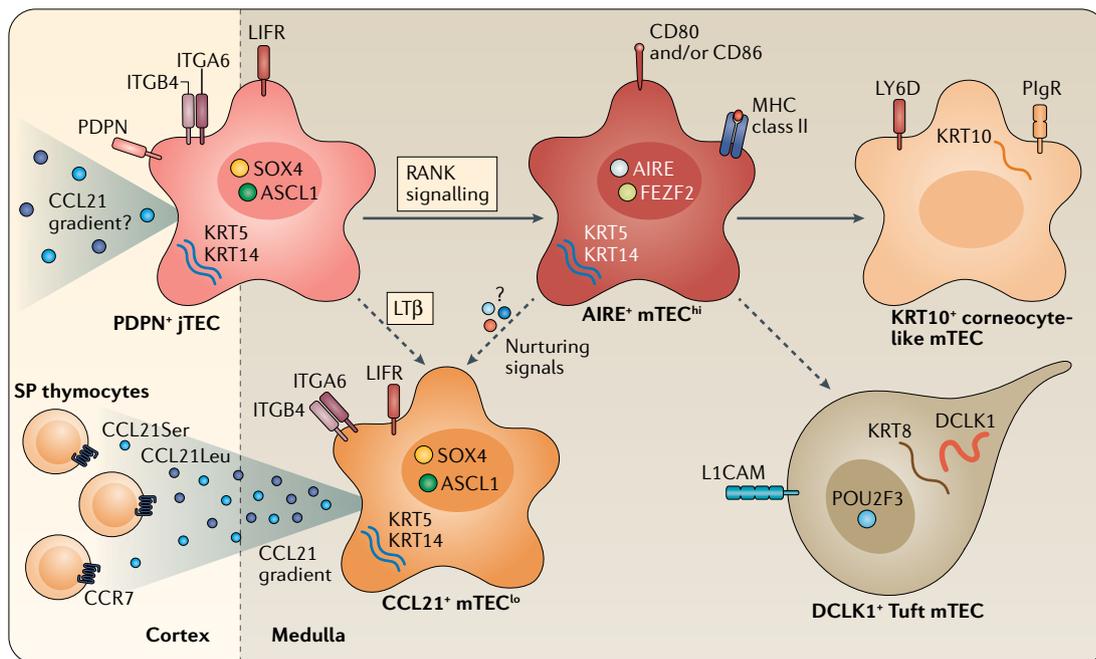


Fig. 3 | Model for the differentiation of medullary thymic epithelial cells. Several lines of evidence suggest that in the adult mouse thymus, PDPN⁺ junctional thymic epithelial cells (jTECs), which are localized at the cortico-medullary junction, are the earliest subset of medullary thymic epithelial cells (mTECs) and give rise to other mTEC subsets including CCL21⁺ mTEC^{lo} cells and AIRE⁺ mTEC^{hi} cells. The differentiation into AIRE⁺ cells is mainly driven by receptor activator of nuclear factor- κ B (RANK) signalling, and the differentiation into CCL21⁺ mTECs was shown to require lymphotoxin- β (LT β). The CCL21⁺ mTECs were shown to facilitate recruitment of positively selected single-positive (SP) CCR7⁺ thymocytes into the medulla through the secretion of CCL21Ser. AIRE⁺ mTECs were found to differentiate further into KRT10⁺ corneocyte-like mTECs and possibly also into DCLK1⁺ thymic tuft cells (tuft mTECs). Although the PDPN⁺ jTECs and CCL21⁺ mTECs are not developmentally derived from AIRE⁺ mTECs, their cellularity is markedly reduced in AIRE-deficient thymi, which suggests that their survival and/or proliferation are regulated by their AIRE⁺ counterparts. Both PDPN⁺ jTECs and CCL21⁺ mTECs share expression of several key genes, including *Ccl21a*, *Ccl21c*, *Itga6*, *Itgb4*, *Krt5*, *Krt14*, *Lifr*, *Sox4* and *Ascl1*. AIRE, autoimmune regulator; CCL21, CC-chemokine ligand 21; CCR7, CC-chemokine receptor 7; FEZF2, Fez family zinc finger protein 2; KRT5, keratin type II cytoskeletal 5; KRT8, keratin type II cytoskeletal 8; KRT10, keratin type I cytoskeletal 10; KRT14, keratin type I cytoskeletal 14; L1CAM, neural cell adhesion molecule L1; PDPN, podoplanin; PlgR, polymeric immunoglobulin receptor.

CCL21Ser-deficient mice
A transgenic mouse model in which the *tdTomato* reporter gene is inserted at the translation initiation site of the *Ccl21a* gene promoter, without affecting the expression of *Ccl21b* or *Ccl21c* genes. Mice homozygous for the insertion are therefore specifically deficient for CCL21Ser, but not for CCL21Leu, which is encoded by *Ccl21b* and/or *Ccl21c*.

Tissue-restricted antigens (TRAs). Proteins that are expressed, processed and presented by thymic epithelial cells to developing thymocytes for the purpose of selection that are otherwise specifically expressed only in five or fewer peripheral tissues.

model and/or to provide additional molecular markers that would enable separation of the putative progenitors from the other CCL21-expressing mTECs. It is also worth mentioning that the individual CCR7 ligands may have diverse and non-redundant roles, as shown by CCL21Ser-deficient mice, which have impaired accumulation of positively selected thymocytes in the thymic medulla and defective establishment of central tolerance⁷ or impaired formation of the intrathymic conventional dendritic cell 1 (cDC1) subset⁷¹.

Mature AIRE-expressing mTECs

The maturation of mTECs is phenotypically defined by the concomitant upregulation of several key genes, including those encoding MHC class II, CD80, AIRE, and AIRE-dependent and AIRE-independent tissue-restricted antigens (TRAs)^{27,66}. The mature AIRE⁺ mTEC^{hi} population is characterized by a high degree of intrinsic heterogeneity, which is essential for the role of these cells in both negative selection and agonist selection of self-reactive thymocyte clones (reviewed in REF.⁷²) (FIG. 4a).

In order to carry out these functions, mature mTECs must express and present developing thymocytes with

the complete array of self-proteins and peptides that they might encounter once they are released into the periphery, as failure to express even a single peptide during this intricate selection process could result in devastating autoimmunity⁷³⁻⁷⁸. To this end, the mature mTEC population expresses up to 90% of the entire coding genome, which is a considerably higher proportion than most other cell types⁷⁹. Moreover, to maximize the exposure of thymocytes to diverse protein isoforms, mTECs are equipped with extensive mechanisms for alternative splicing and RNA editing^{80,81}. The phenomenon of promiscuous gene expression (PGE) of hundreds of tissue-restricted genes at the population level is an intrinsic property of mTECs⁸². It is readily detected in the mouse embryo as early as embryonic day 16-17 with the emergence of mature AIRE⁺ mTECs and has been shown to increase in complexity until birth⁸³⁻⁸⁶. Interestingly, when PGE is subtracted from the gene signature of mature mTEC^{hi} cells, their internal heterogeneity almost disappears, which suggests that this heterogeneity primarily stems from the differential expression of TRAs at a single-cell level⁷⁹. Indeed, it has been evident for more than two decades that, at the

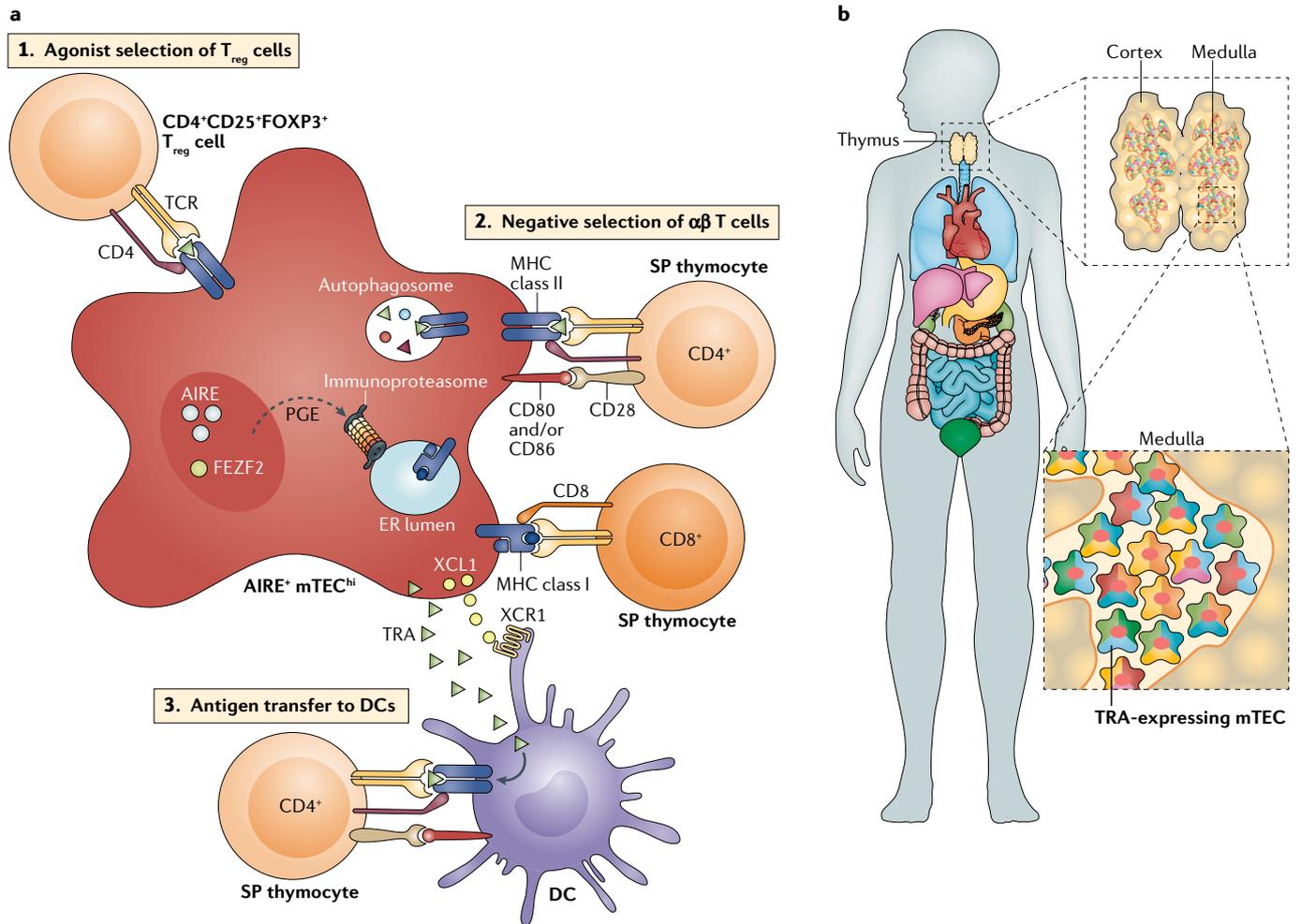


Fig. 4 | The heterogeneity of mature medullary thymic epithelial cells stems from differential expression of tissue-restricted antigens. a Mature medullary thymic epithelial cells (mTECs) facilitate either agonist selection (1) or negative selection (2) of self-reactive thymocytes. To this end, mTECs are characterized by promiscuous gene expression (PGE) of thousands of tissue-restricted antigens (TRAs), which is mainly orchestrated by autoimmune regulator (AIRE) and to some extent by Fez family zinc finger protein 2 (FEZF2). Mature mTECs also upregulate expression of costimulatory molecules, such as CD80 and CD86, and of MHC class II, which is required for presentation of self-antigens. Antigen presentation on MHC class II molecules is mediated by high and constitutive levels of autophagy in mature mTECs, as well as by immunoproteasome activity. Ultimately, self-antigens are presented to single-positive (SP) thymocytes either directly by mature mTECs or indirectly by antigen transfer to dendritic cells (DCs) (3), which express the chemokine receptor XCR1 and are attracted to the thymus by AIRE-dependent expression of XCL1. **b** At a population level, mTECs express ~90% of the self-proteome, such that the antigen repertoire in the thymus medulla mirrors the highly diverse and tissue-specific repertoire (represented by different colours) of the entire body. However, at the single-cell level, each individual mTEC expresses only a fraction of the entire antigen ‘inventory’, which suggests that a mosaic coverage is required to achieve maximal selection efficiency. It has been shown in several studies that AIRE-driven gene expression of TRAs by mTECs follows rules of ‘ordered stochasticity’, such that certain genes tend to be co-expressed in an individual mTEC. ER, endoplasmic reticulum; TCR, T cell receptor; T_{reg} cell, regulatory T cell.

single-cell level, not all mature mTECs express all TRAs. Specifically, early studies based on microscopic analysis of the thymus showed that only a few cells in a given thymic section stain positively for TRAs such as insulin, somatostatin or retinol-binding protein 3 (REFS^{87,88}). In addition, single-cell PCR of mature mTECs of both mouse and human origin showed that each individual mTEC expresses only a limited set of TRAs and an individual TRA is expressed by only 0.4–3% of cells^{89–93}. Therefore, the complete expression of all TRAs by the mTEC population must be owing to the summation of

mosaic expression of TRAs by individual mTECs (FIG. 4b). Moreover, the expression of TRAs in these studies was suggested to be stochastic, as the TRAs expressed in individual mTECs did not show any discernible pattern, even within a defined locus. This expression of TRAs by mTECs also differed significantly from the expression pattern found in peripheral cell lineages that normally express the TRAs, as seen by discrepancies in terms of monoallelic versus biallelic transcription, as well as the use of different transcription start sites and transcription factors^{90,91}. The stochastic nature of this process makes

intuitive sense as it functions as the initial and most substantial system of checks and balances of the TCR repertoire, which is itself generated in a semi-random manner through VDJ recombination. Furthermore, the restricted expression of a limited number of TRAs per single mTEC is essential to increase the density of the derived epitopes that are presented to maturing thymocytes on the limited number of MHC class II molecules, such that effective selection may be achieved^{91,93,94}.

The advent of high-throughput single-cell technologies has enabled a detailed examination of the expression pattern of TRAs per single mTEC at high resolution. This has provided important insights into the molecular intricacies of the PGE process and has helped to correct some inaccurate interpretations based on gene expression profiling of the bulk and heterogeneous mature mTEC population. Deep RNA sequencing of ~200 individual mature mTECs showed that up to 95% of TRAs are represented in such a small sample size, which suggests that the number of mature mTECs needed to interact with a developing thymocyte in order to cover the entire TRA library is rather small^{79,80,94}. When the repertoire of TRAs per cell was examined, a given AIRE-dependent gene was expressed in only ~1% of mature mTECs, compared with ~2% of mTECs for AIRE-enhanced genes and 9% of mTECs for AIRE-independent genes, whereas all other genes were expressed in ~33% of all mature mTECs⁷⁹. Interestingly, although a given AIRE-dependent TRA is expressed in only a very small fraction of mTECs, its expression level in the corresponding single cell is very high, although significantly lower than in the peripheral tissue in which it is normally expressed^{179,80,90,94,95}. Despite the apparent stochastic nature of TRA expression highlighted by the scRNA-seq analyses^{79,80}, there do seem to be some rules guiding this process. For example, it was shown that many TRA genes preferentially fall into co-expression clusters, which suggests that PGE in specific individuals follows rules of ordered stochasticity (FIG. 4b) but has high inter-individual variance⁸⁰. In a parallel study, another group found evidence of overlap between co-expression clusters and suggested that they could potentially be placed on a continuum of TRA expression, with mTECs transitioning between different co-expression clusters throughout their lifespan and perhaps covering the entire TRA repertoire over time⁹⁴. Experimental evidence to validate that a single mTEC is capable of expressing most of the TRA gene repertoire during its lifetime has yet to be provided and we still lack spatial information that could potentially tie together seemingly unrelated cells.

The mechanism by which mature mTECs bring about this unique gene expression programme has been only partly elucidated and marks another level of heterogeneity within this population, as two factors — namely, AIRE⁷⁴ and Fez family zinc finger protein 2 (FEZF2)⁹⁶ — have been implicated in inducing PGE. AIRE, which is the sole gene responsible for autoimmune polyendocrine syndrome type 1 (APS1; also known as autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED))^{97,98}, has been the focus of many studies over the past two decades and has been extensively reviewed elsewhere^{4,72,99}. Importantly,

AIRE is expressed by approximately half of the mature mTEC^{hi} population, in which it has been shown to regulate the expression of ~4,000 genes, most of which are TRAs^{74,79,80,91}. FEZF2 has been reported to upregulate the expression of ~400 AIRE-independent genes and thereby to complement the role of AIRE in PGE and the subsequent negative selection and/or agonist selection of self-reactive thymocytes⁹⁶. However, it is important to stress that many of the FEZF2-induced TRA genes that were reported in the original study to be AIRE independent (such as *Krt10*, *Klk1b16*, *Apoc2*, *Cyp24a1* and *Muc3*)⁹⁶ have been identified by other groups as AIRE-dependent genes^{64,79–81}. Therefore, although FEZF2 seems to be an important factor with the potential to shape the mTEC-specific gene signature, the number of AIRE-independent TRA genes that are induced by FEZF2 is still a matter of debate and may be markedly lower than that proposed by REF.⁹⁶.

Terminally differentiated mTECs

Until relatively recently, the AIRE⁺ mTEC^{hi} population was regarded as the final stage of mTEC development⁶⁶. However, over the past several years, a growing body of evidence has shown that the AIRE⁺ mTEC^{hi} cells continue to differentiate and, consequently, give rise to additional mTEC subsets^{6,10–12,67,68,100}, including corneocyte-like mTECs and thymic tuft cells^{5,9,13} (FIG. 3).

Corneocyte-like mTECs. Probably the first experimental evidence to suggest that AIRE promotes mTEC differentiation was provided by a study showing that AIRE-deficient mice have reduced numbers of KRT10⁺ mTECs and impaired formation of Hassall's corpuscles in their thymi¹⁰. Moreover, a subsequent study showed that the development of these KRT10⁺ mTECs, and the subsequent formation of Hassall's corpuscles, comes after the development of AIRE⁺ mTECs during ontogeny and is regulated by the lymphotoxin signalling pathway¹¹. These findings were further supported by several additional studies based on in vivo fate-mapping experiments, which showed that AIRE⁺ mTEC^{hi} cells continue their differentiation programme by downregulation of AIRE and MHC class II expression and concomitant upregulation of expression of various genes, such as those encoding keratin type II cytoskeletal 1 (KRT1), keratin type I cytoskeletal 10 (KRT10), involucrin, desmogleins and serine protease inhibitor Kazal-type 5 (SPINK5)^{12,68,69,100–102}. Interestingly, all of these genes are also highly specific to terminally differentiated keratinocytes (corneocytes) that compose the outermost layer of the epidermis (stratum corneum) (FIG. 5a). To function as an effective physical barrier against the external environment, corneocytes lose their nuclei and cytosolic organelles and acquire a tough outer cell envelope composed of aggregated keratins. Similarly to corneocytes, the post-AIRE KRT10⁺ mTEC^{lo} cells also lose their nuclei as they become Hassall's corpuscles¹² (FIG. 5a). Moreover, their striking similarity to corneocytes is further supported by several recent transcriptomic studies showing that, in addition to keratins (such as *Krt1*, *Krt7*, *Krt10* and *Krt77*), desmogleins and involucrin, the corneocyte-like mTECs (also known as mTEC III cells)⁵ also express

AIRE-dependent gene

A gene that requires autoimmune regulator (AIRE) for its expression.

AIRE-enhanced genes

Genes that have low levels of expression in the absence of autoimmune regulator (AIRE) but expression of which is significantly increased by AIRE.

Ordered stochasticity

The autoimmune regulator (AIRE) protein is said to operate with ordered stochasticity, such that the genes it activates in individual medullary thymic epithelial cells are stochastically selected, but the process is not completely random as co-expression groups of genes within cells are found.

Autoimmune polyendocrine syndrome type 1

(APS1). A genetic disorder, caused by mutations in the *AIRE* gene, that leads to a devastating multi-organ autoimmune syndrome. It is diagnosed when patients present with at least two out of three of the classical symptoms, which include chronic mucocutaneous candidiasis, hypoparathyroidism and adrenocortical insufficiency.

Hassall's corpuscles

Islet-like structures found in the medullary region of the thymus that are composed of squamous epithelial cells expressing high levels of various keratins (for example, KRT10) and involucrin.

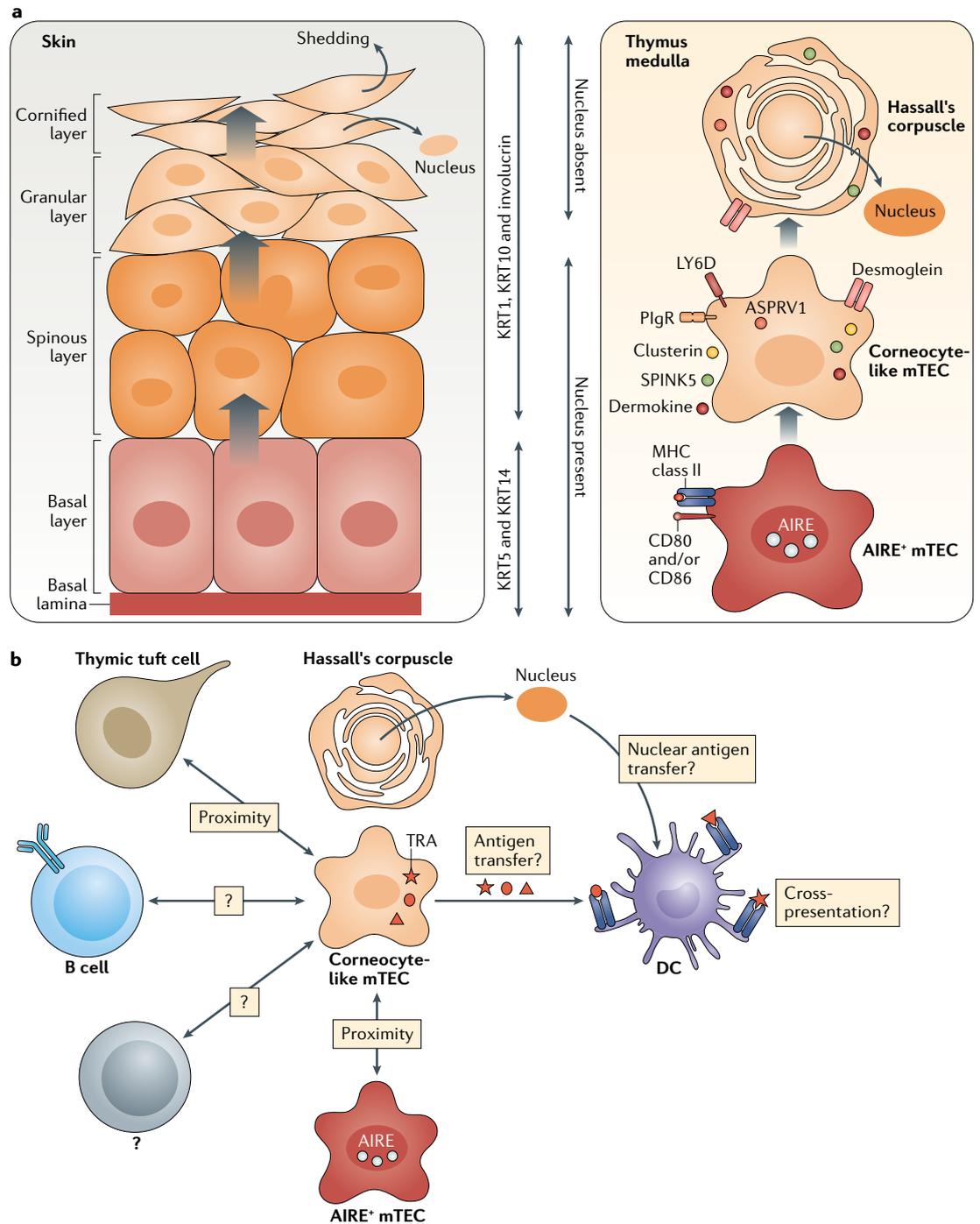


Fig. 5 | Corneocyte-like medullary thymic epithelial cells and Hassall's corpuscles. **a** | The terminal differentiation of AIRE⁺ medullary thymic epithelial cells (mTECs) (right panel) is highly similar to skin keratinocyte differentiation (left panel). The differentiation of mTECs is accompanied by downregulation of autoimmune regulator (AIRE) and MHC class II expression and a concomitant upregulation of expression of LY6D and/or polymeric immunoglobulin receptor (PlgR), as well as of various genes with high specificity for terminally differentiated keratinocytes (corneocytes), such as the genes encoding keratin type II cytoskeletal 1 (KRT1) and keratin type I cytoskeletal 10 (KRT10), involucrin, desmogleins, clusterin, dermokine, serine protease inhibitor Kazal-type 5 (SPINK5) and retroviral-like aspartic protease 1 (ASPRV1). Further similarities between corneocyte-like mTECs and corneocytes include the loss of nuclei and cornification as corneocyte-like mTECs differentiate into Hassall's corpuscles. **b** | The functional roles of corneocyte-like mTECs and/or of Hassall's corpuscles are still poorly understood. As corneocyte-like mTECs downregulate MHC class II expression but still express many AIRE-dependent and AIRE-independent tissue-restricted antigens (TRAs), they may potentially transfer these antigens to dendritic cells (DCs) for subsequent cross-presentation. The Hassall's corpuscles and/or corneocyte-like mTECs were found to be surrounded by several other cell subsets in the medulla, including AIRE⁺ mTECs, thymic B cells and, perhaps, additional cell types; however, the functional relevance of these physical associations has yet to be determined.

clusterin, dermokine, retroviral-like aspartic protease 1 (*Asprv1*), cystatin A and other corneocyte-specific genes^{5,13}. Therefore, these data collectively suggest that the terminal development of mTECs highly resembles that of epidermal keratinocytes, where cornification functions as an alternative route to cell death⁶⁷ (FIG. 5a).

The specific surface markers that would enable the physical isolation and detailed characterization of these post-AIRE corneocyte-like mTECs remained unknown for a long time. Recently, it was shown that these cells can be separated from other mTEC subsets based on staining with *Tetragonolobus purpureus* agglutinin (TPA)⁶⁷, a lectin that was previously shown to specifically mark Hassall's corpuscles¹⁰³. More recently, the corneocyte-like mTECs were found to express high levels of LY6D and/or of the gene encoding polymeric immunoglobulin receptor (*Ptgr*) (FIG. 5a), whereas they did not express ITGB4 (or *Itga6*), which are characteristic of mTEC I cells⁵. Therefore, the combination of these markers now enables effective separation of corneocyte-like mTECs from the other mTEC^{lo} subsets. Interestingly, scRNA-seq analysis of TECs throughout ontogeny showed that the corneocyte-like mTECs cannot be detected in mouse embryonic thymi and appear only later after birth⁵. This is in line with previous studies showing that post-AIRE mTECs appear only after the emergence of their AIRE⁺ precursors^{11,67} and that they still express many AIRE-dependent genes, despite the downregulation of AIRE itself.

The exact functional roles of the corneocyte-like mTECs and/or of the Hassall's corpuscles are still poorly understood. The downregulation of MHC class II expression suggests that they are not likely to be efficient at direct antigen presentation to CD4⁺ T cells. However, the fact that they still express a large fraction of AIRE-dependent and AIRE-independent TRA genes suggests that they may still be involved in the induction of immunological tolerance, for example by passing their antigen cargo to dendritic cells (DCs) for subsequent cross-presentation to thymocytes. Although there is a strong body of evidence that AIRE⁺ mTECs actively transfer a fraction of their self-antigen repertoire to DCs^{104–106} (FIG. 4a), it is unclear whether the corneocyte-like mTECs also use such an antigen-transfer mechanism (FIG. 5b). In support of this hypothesis, it was shown that Hassall's corpuscles in the human thymus express thymic stromal lymphopoietin (TSLP), which can subsequently induce expression of the costimulatory molecules CD80 and CD86 in thymus-resident DCs. Moreover, these TSLP-conditioned DCs could induce FOXP3⁺ T_{reg} cells¹⁰⁷, which suggests that Hassall's corpuscles might have an important role in tolerance induction. Interestingly, however, corneocyte-like mTECs in the mouse thymus do not seem to express TSLP⁵ and nor do they seem to be associated with FOXP3⁺ T_{reg} cells and/or DCs¹⁰⁸. Given that Hassall's corpuscles are morphologically defined cellular islands that are scattered throughout the thymic medulla, it is likely that their primary role is to create local cellular microenvironments that provide specific signals for other cell types. Indeed, it has been shown by several independent studies that Hassall's corpuscles and/or corneocyte-like mTECs are primarily surrounded by other cell

subsets, including AIRE⁺ mTECs¹¹, thymic tuft cells¹³ or thymic B cells¹⁰⁹. However, the functional relevance of these physical associations remains elusive (FIG. 5b).

Thymic tuft cells. The other main subset of terminally differentiated mTECs that was recently identified in both mouse and human thymi comprises thymic tuft cells^{5,13–15}. Tuft (or brush) cells are flask-shaped epithelial cells with chemosensory properties that were originally identified in mucosal tissues owing to their characteristic tuft-like microvilli on their apical sides^{110,111}. Interestingly, cells with some morphological features similar to those of tuft cells were described in the thymus as early as three decades ago^{112,113}, but a population of thymic tuft-like cells was only recently identified and characterized^{5,13–15}. This discovery suggests that tuft cells are not exclusive to mucosal and polarized tissues but might also have an important role in primary lymphatic organs, such as the thymus.

The development of thymic tuft cells is, similar to their mucosal counterparts and unlike other TEC subsets, controlled by the transcription factor POU2F3 (REFS^{5,13,114–116}). Moreover, during ontogeny, both mucosal tuft cells^{117–120} and thymic tuft cells⁵ are found only during late embryonic development or after birth. Importantly, lineage tracing experiments carried out by two independent groups have suggested that whereas more than half of the thymic tuft cells arise from mature mTEC^{hi} cells^{5,13}, the remaining 40–50% do not seem to pass through the AIRE-expressing mTEC stage. Specifically, diphtheria toxin treatment of mice in which diphtheria toxin receptor (DTR) expression is driven by the *Aire* promoter resulted in only a partial (yet significant) depletion of thymic tuft cells¹³. Furthermore, AIRE deficiency does not result in marked changes in thymic tuft cell development and/or their gene expression profile⁵. Finally, scRNA-seq and chromatin immunoprecipitation followed by sequencing (ChIP-seq) analyses show that thymic tuft cells are very distant from AIRE⁺ mTECs in terms of both their transcription profile and their epigenetic landscape, with no clear cell subset that could be a putative transitional state between the two⁵. Therefore, additional studies are required to better delineate the mechanisms underlying tuft cell development in the thymus and their relationship to mature mTECs.

In addition to their unique morphological features, tuft cells are characterized by a unique gene expression signature^{121,122}. Both mucosal and thymic tuft cells express serine/threonine protein kinase DCLK1, which is one of the most commonly used markers for these cells^{5,13,118}, as well as several key genes that are involved in the biosynthesis of various secreted molecules with diverse biological functions. For example, they express high levels of IL-25 or genes encoding enzymes involved in acetylcholine synthesis (such as *ChAT*) or in prostaglandin and leukotriene synthesis (such as *Ptgs1*, *Hpgds* and *Alox5*) (FIG. 6). The capacity of tuft cells to express these specific genes probably reflects their putative biological roles in regulating immune responses and/or tissue homeostasis¹²³. In keeping with their mucosal counterparts, thymic tuft cells express genes such as *Plcb2*, *Trpm5*, *Gnb3* and *Gng13*, which are involved in

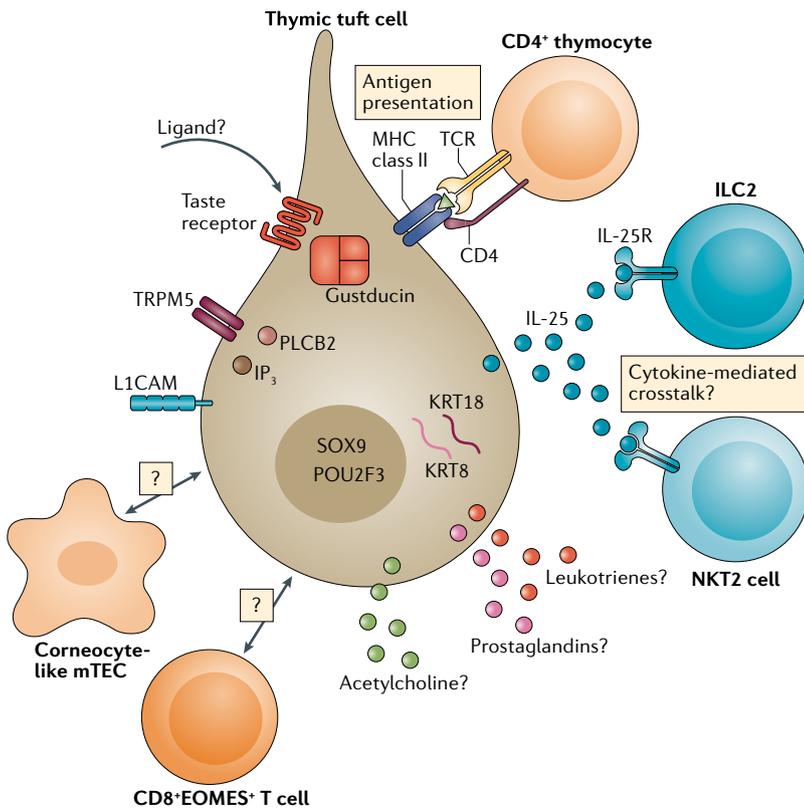


Fig. 6 | Thymic tuft cells. Thymic tuft cells are chemosensory cells whose development depends on the transcription factor POU2F3. They have been shown to express neural cell adhesion molecule L1 (L1CAM), in addition to keratin type II skeletal 8 (KRT8), keratin type I cytoskeletal 18 (KRT18) and the transcription factor SOX9. They also express taste receptors and components of their downstream signalling cascade (such as gustducin, 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase β 2 (PLCB2), inositol-1,4,5-trisphosphate (IP_3) and TRPM5), although the signals to which thymic tuft cells respond are currently unknown. Thymic tuft cells also express key enzymatic machineries for the biosynthesis of various biologically active molecules, including acetylcholine, leukotrienes and prostaglandins, which suggests that they can affect the homeostasis of neighbouring cells. Similarly to their mucosal tuft cell counterparts, thymic tuft cells express high levels of IL-25, which can act on cells expressing IL-25 receptor (IL-25R), including group 2 innate lymphoid cells (ILC2s) and type 2 natural killer T (NKT2) cells. Indeed, the absence of tuft cells in *Pou2f3*-knockout mice resulted in increased numbers of thymic ILC2s and decreased numbers of thymic and splenic NKT2 cells and thymic EOMES⁺CD8⁺ T cells. Thymic tuft cells were shown to localize next to corneocyte-like medullary thymic epithelial cells (mTECs), although the nature of this proximity has yet to be elucidated. TCR, T cell receptor.

Type 2 immune response
An immune response characterized by an increased production of various cytokines (such as IL-4, IL-5 and IL-13) and concomitant activation of distinct immune cell populations, including T helper 2 cells, eosinophils, basophils, mast cells, group 2 innate lymphoid cells and type 2 natural killer T cells. The type 2 immune response has an important role in host defence against parasites, but when dysregulated may underlie the development of diverse allergic disorders.

the taste reception signalling pathway. Although it is not clear why tuft cells express such genes, it is probably linked to their natural chemosensory capacity. Despite these striking molecular similarities between thymic tuft cells and mucosal tuft cells, there are also several clear differences. Indeed, a recent RNA-seq analysis compared tuft cells isolated from different tissues and found that the transcriptional signature of thymic tuft cells was the most distinct¹²⁴. Unlike other types of tuft cell, thymic tuft cells are characterized by relatively high levels of expression of several members of the taste receptor 2 (*Tas2r*) locus (which encodes a family of receptors for bitter taste), *Gnat3*, *L1cam* or genes encoding MHC class II and CD74, which are related to antigen presentation^{5,13} (FIG. 6). By contrast, thymic tuft cells do not express

various genes that are characteristic of intestinal tuft cells (such as *Lgals2*, *Lgals4*, *Muc13*, *Fabp1* and *Apoa1*).

Importantly, based on their relatively high levels of expression of genes encoding the antigen presentation machinery, their medullary localization and expression of various TRA genes, it has been hypothesized that thymic tuft cells may be involved in the induction of immunological tolerance¹³. Indeed, IL-25 immunization of mice transplanted with wild-type or tuft cell-deficient thymi showed that the presence of tuft cells was essential to prevent the generation of IL-25-specific antibodies¹³. However, it is important to stress that although thymic tuft cells express MHC class II molecules, their expression levels are more than an order of magnitude lower than those of AIRE⁺ mTECs, which suggests that tuft cells are not likely to be very potent as antigen-presenting cells. In addition, unlike AIRE⁺ mTECs, thymic tuft cells do not have any features of PGE⁵. Finally, it is also not clear why a tuft cell-specific gene signature would preferentially be selected for tolerance induction in the thymus over other tissue-specific and/or cell-specific antigens. Therefore, further experiments in more physiological settings are required to determine whether tolerance induction is indeed the primary role of thymic tuft cells or whether they might have alternative and/or additional roles.

Several independent studies recently showed that mouse intestinal tuft cells are the prime activators of the type 2 immune response to parasitic infection and are essential for parasite clearance^{114,124–128}. To this end, they secrete pre-synthesized IL-25 in response to parasites, which in turn activates gut-resident group 2 innate lymphoid cells (ILC2s), eventually resulting in the initiation and amplification of the type 2 cytokine response with subsequent parasite clearance. As thymic tuft cells also express high basal levels of IL-25, it has been speculated that they may also promote a type 2 response in the thymus. Indeed, tuft cell deficiency is associated with reduced numbers of type 2 natural killer T (NKT2) cells and EOMES⁺CD8⁺ single-positive thymocytes in the thymus¹³, and by increased frequencies and numbers of thymic ILC2s⁵. However, the actual role of the type 2 immune response in the thymus, and in particular the role of thymic NKT2 cells and/or ILC2s, is still largely unclear (FIG. 6). Correspondingly, it remains to be determined whether and how thymic tuft cells activate a type 2 immune response and, most importantly, why such a response is required in a non-mucosal organ that is not exposed to parasites.

Concluding remarks

Recent studies have provided clear evidence that the division of TECs into cTECs and mTECs is insufficient, as each of these two main TEC populations is, in fact, highly heterogeneous and comprises multiple subpopulations with distinct developmental, molecular, morphological and functional characteristics.

Although recent years have seen tremendous progress in a comprehensive decomposition and detailed molecular characterization of the TEC compartment, including the identification of thymic tuft cells and corneocyte-like mTECs, an understanding of their exact functional roles and physiological relevance remains largely elusive.

Group 2 innate lymphoid cells (ILC2s). A population of lymphoid-derived cells that are defined by the absence of key lymphoid, myeloid and dendritic cell markers and by expression of the transcription factor GATA3 and various type 2 cytokines, such as IL-4, IL-5, IL-9 and IL-13. They have been identified in many tissues, including the skin, intestinal tract and respiratory tract, and they have been suggested to have a role in immune responses against parasites, as well as in allergy and asthma.

Therefore, the challenges in the future will likely revolve around the functional characterization of these TEC subsets and elucidating their interactions with other cell types resident in the thymus. Corneocyte-like mTECs are likely to complement AIRE⁺ mTECs in the induction of central tolerance, whereas thymic tuft cells might, owing to their putative cholinergic and chemosensory capacities, have other functional roles that are unrelated to tolerance induction. An additional challenge will be to map the developmental paths taken by different TEC subpopulations and further delineate and validate their developmental hierarchy. This will include resolving the controversy regarding the identity of the unipotent and/or bipotent TEPCs and uncovering the differentiation pathways that lead to the generation of mature

antigen-presenting mTECs and cTECs, corneocyte-like TECs and thymic tuft cells. Therefore, a key prerequisite to successfully address these questions will likely be the identification of the specific signals and/or transcription factors that are crucial for the development of the individual TEC subsets. An additional point to be addressed is the elucidation of the complex molecular mechanisms used by AIRE and FEZF2 to mediate PGE for the induction of central tolerance. Finally, our still limited understanding of cTEC heterogeneity, as well as of the functional relevance of TNCs, begs for additional cutting-edge studies based on single-cell genomics and/or imaging in the future.

Published online 5 December 2019

- Klein, L., Kyewski, B., Allen, P. M. & Hogquist, K. A. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat. Rev. Immunol.* **14**, 377–391 (2014).
- Aschenbrenner, K. et al. Selection of Foxp3⁺ regulatory T cells specific for self antigen expressed and presented by Aire⁺ medullary thymic epithelial cells. *Nat. Immunol.* **8**, 351–358 (2007). **This paper presents one of the first key pieces of evidence to show that AIRE⁺ mTECs are not only involved in clonal deletion but also have a key role in the induction of FOXP3⁺ T_{reg} cells specific for tissue-restricted antigens.**
- Cowan, J. E. et al. The thymic medulla is required for Foxp3⁺ regulatory but not conventional CD4⁺ thymocyte development. *J. Exp. Med.* **210**, 675–681 (2013).
- Takahama, Y., Ohigashi, I., Baik, S. & Anderson, G. Generation of diversity in thymic epithelial cells. *Nat. Rev. Immunol.* **17**, 295–305 (2017).
- Bornstein, C. et al. Single-cell mapping of the thymic stroma identifies IL-25-producing tuft epithelial cells. *Nature* **559**, 622–626 (2018). **This study provides the first comprehensive cell atlas for the thymic stroma (based on scRNA-seq analysis) and identifies thymic tuft cells as a highly divergent subset of mTECs.**
- Lkhagvasuren, E., Sakata, M., Ohigashi, I. & Takahama, Y. Lymphotoxin β receptor regulates the development of CCL21-expressing subset of postnatal medullary thymic epithelial cells. *J. Immunol.* **190**, 5110–5117 (2013).
- Kozai, M. et al. Essential role of CCL21 in establishment of central self-tolerance in T cells. *J. Exp. Med.* **214**, 1925–1935 (2017).
- Onder, L. et al. Alternative NF-κB signaling regulates mTEC differentiation from podoplanin-expressing precursors in the cortico-medullary junction. *Eur. J. Immunol.* **45**, 2218–2231 (2015).
- Miragaia, R. J. et al. Single-cell RNA-sequencing resolves self-antigen expression during mTEC development. *Sci. Rep.* **8**, 685 (2018).
- Yano, M. et al. Aire controls the differentiation program of thymic epithelial cells in the medulla for the establishment of self-tolerance. *J. Exp. Med.* **205**, 2827–2838 (2008).
- White, A. J. et al. Lymphotoxin signals from positively selected thymocytes regulate the terminal differentiation of medullary thymic epithelial cells. *J. Immunol.* **185**, 4769–4776 (2010). **Together with Yano et al. (2008), this study is the first to show that AIRE⁺ mTECs are not terminally differentiated cells but rather give rise to phenotypically distinct subsets characterized by high levels of KRT10 expression.**
- Wang, X. et al. Post-Aire maturation of thymic medullary epithelial cells involves selective expression of keratinocyte-specific autoantigens. *Front. Immunol.* **3**, 19 (2012).
- Miller, C. N. et al. Thymic tuft cells promote an IL-4-enriched medulla and shape thymocyte development. *Nature* **559**, 627–631 (2018). **This study reports the identification and characterization of thymic tuft cells.**
- Panneck, A. R. et al. Cholinergic epithelial cell with chemosensory traits in murine thymic medulla. *Cell Tissue Res.* **358**, 737–748 (2014).
- Soultanova, A. et al. Cholinergic chemosensory cells of the thymic medulla express the bitter receptor Tas2r131. *Int. Immunopharmacol.* **29**, 143–147 (2015).
- Ulyanchenko, S. et al. Identification of a bipotent epithelial progenitor population in the adult thymus. *Cell Rep.* **14**, 2819–2832 (2016).
- Wong, K. et al. Multilineage potential and self-renewal define an epithelial progenitor cell population in the adult thymus. *Cell Rep.* **8**, 1198–1209 (2014).
- Ucar, A. et al. Adult thymus contains FoxN1⁺ epithelial stem cells that are bipotent for medullary and cortical thymic epithelial lineages. *Immunity* **41**, 257–269 (2014).
- Lepletier, A. et al. Interplay between follistatin, activin A, and BMP4 signaling regulates postnatal thymic epithelial progenitor cell differentiation during aging. *Cell Rep.* **27**, 3887–3901 (2019).
- Mayer, C. E. et al. Dynamic spatio-temporal contribution of single β5t⁺ cortical epithelial precursors to the thymus medulla. *Eur. J. Immunol.* **46**, 846–856 (2016).
- Ohigashi, I. et al. Adult thymic medullary epithelium is maintained and regenerated by lineage-restricted cells rather than bipotent progenitors. *Cell Rep.* **13**, 1432–1443 (2015).
- Rossi, S. W., Jenkinson, W. E., Anderson, G. & Jenkinson, E. J. Clonal analysis reveals a common progenitor for thymic cortical and medullary epithelium. *Nature* **441**, 988–991 (2006).
- Bleul, C. C. et al. Formation of a functional thymus initiated by a postnatal epithelial progenitor cell. *Nature* **441**, 992–996 (2006). **Together with Rossi et al. (2006), this paper provides the first evidence to support the existence of bipotent TEPCs in the embryonic and neonatal thymus.**
- Ohigashi, I. et al. Aire-expressing thymic medullary epithelial cells originate from β5t-expressing progenitor cells. *Proc. Natl Acad. Sci. USA* **110**, 9885–9890 (2013).
- Bennett, A. R. et al. Identification and characterization of thymic epithelial progenitor cells. *Immunity* **16**, 803–814 (2002).
- Gill, J., Malin, M., Hollander, G. A. & Boyd, R. Generation of a complete thymic microenvironment by MTS24⁺ thymic epithelial cells. *Nat. Immunol.* **3**, 635–642 (2002).
- Rossi, S. W. et al. Redefining epithelial progenitor potential in the developing thymus. *Eur. J. Immunol.* **37**, 2411–2418 (2007).
- Baik, S., Jenkinson, E. J., Lane, P. J. L., Anderson, G. & Jenkinson, W. E. Generation of both cortical and Aire⁺ medullary thymic epithelial compartments from CD205⁺ progenitors. *Eur. J. Immunol.* **43**, 589–594 (2013).
- Alves, N. L. et al. Serial progression of cortical and medullary thymic epithelial microenvironments. *Eur. J. Immunol.* **44**, 16–22 (2014).
- Dumont-Lagacé, M. et al. Detection of quiescent radioresistant epithelial progenitors in the adult thymus. *Front. Immunol.* **8**, 1717 (2017).
- Sheridan, J. M. et al. Thymospheres are formed by mesenchymal cells with the potential to generate adipocytes, but not epithelial cells. *Cell Rep.* **21**, 934–942 (2017).
- Barsanti, M. et al. A novel Foxn1^{creGFP/+} mouse model identifies Bmp4-induced maintenance of Foxn1 expression and thymic epithelial progenitor populations. *Eur. J. Immunol.* **47**, 291–304 (2017).
- Anderson, G. & Takahama, Y. Thymic epithelial cells: working class heroes for T cell development and repertoire selection. *Trends Immunol.* **33**, 256–263 (2012).
- Plotkin, J., Prockop, S. E., Lepique, A. & Petrie, H. T. Critical role for CXCR4 signaling in progenitor localization and T cell differentiation in the postnatal thymus. *J. Immunol.* **171**, 4521–4527 (2003).
- Jenkinson, W. E. et al. Chemokine receptor expression defines heterogeneity in the earliest thymic migrants. *Eur. J. Immunol.* **37**, 2090–2096 (2007).
- Gossens, K. et al. Thymic progenitor homing and lymphocyte homeostasis are linked via S1P-controlled expression of thymic P-selectin/CCL25. *J. Exp. Med.* **206**, 761–778 (2009).
- Bleul, C. C. & Boehm, T. Chemokines define distinct microenvironments in the developing thymus. *Eur. J. Immunol.* **30**, 3371–3379 (2000).
- Hozumi, K. et al. Delta-like 4 is indispensable in thymic environment specific for T cell development. *J. Exp. Med.* **205**, 2507–2513 (2008).
- Koch, U. et al. Delta-like 4 is the essential, nonredundant ligand for Notch1 during thymic T cell lineage commitment. *J. Exp. Med.* **205**, 2515–2523 (2008).
- Alves, N. L. et al. Characterization of the thymic IL-7 niche in vivo. *Proc. Natl Acad. Sci. USA* **106**, 1512–1517 (2009).
- Ohigashi, I., Kozai, M. & Takahama, Y. Development and developmental potential of cortical thymic epithelial cells. *Immunol. Rev.* **271**, 10–22 (2016).
- Fujimoto, Y. et al. CD83 expression influences CD4⁺ T cell development in the thymus. *Cell* **108**, 755–767 (2002).
- Liu, H. et al. Ubiquitin ligase MARCH 8 cooperates with CD83 to control surface MHC II expression in thymic epithelium and CD4 T cell selection. *J. Exp. Med.* **213**, 1695–1703 (2016).
- von Rohrscheidt, J. et al. Thymic CD4 T cell selection requires attenuation of MARCH8-mediated MHCII turnover in cortical epithelial cells through CD83. *J. Exp. Med.* **213**, 1685–1694 (2016).
- Yang, S. J. et al. The quantitative assessment of MHC II on thymic epithelium: implications in cortical thymocyte development. *Int. Immunol.* **18**, 729–739 (2006).
- Fiorini, E. et al. Cutting edge: thymic crosstalk regulates delta-like 4 expression on cortical epithelial cells. *J. Immunol.* **181**, 8199–8203 (2008).
- Wekerle, H. & Ketelsen, U. P. Thymic nurse cells—la-bearing epithelium involved in T-lymphocyte differentiation? *Nature* **283**, 402–404 (1980).
- Nakagawa, Y. et al. Thymic nurse cells provide microenvironment for secondary T cell receptor a rearrangement in cortical thymocytes. *Proc. Natl Acad. Sci. USA* **109**, 20572–20577 (2012).
- Guyden, J. C. & Pezanno, M. Thymic nurse cells: a microenvironment for thymocyte development and selection. *Int. Rev. Cytol.* **223**, 1–37 (2003).
- Rode, I. & Boehm, T. Regenerative capacity of adult cortical thymic epithelial cells. *Proc. Natl Acad. Sci. USA* **109**, 3463–3468 (2012).
- Akiyama, T., Shinzawa, M. & Akiyama, N. TNF receptor family signaling in the development and functions of medullary thymic epithelial cells. *Front. Immunol.* **3**, 278 (2012).

52. Hamazaki, Y. et al. Medullary thymic epithelial cells expressing Aire represent a unique lineage derived from cells expressing claudin. *Nat. Immunol.* **8**, 304–311 (2007).
53. Sekai, M., Hamazaki, Y. & Minato, N. Medullary thymic epithelial stem cells maintain a functional thymus to ensure lifelong central T cell tolerance. *Immunity* **41**, 753–761 (2014).
54. Baik, S., Sekai, M., Hamazaki, Y., Jenkinson, W. E. & Anderson, G. Relb acts downstream of medullary thymic epithelial stem cells and is essential for the emergence of RANK⁺ medullary epithelial progenitors. *Eur. J. Immunol.* **46**, 857–862 (2016).
55. Akiyama, T. et al. The tumor necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. *Immunity* **29**, 423–437 (2008).
56. Akiyama, T. et al. Dependence of self-tolerance on TRAF6-directed development of thymic stroma. *Science* **308**, 248–251 (2005).
57. Boehm, T., Scheu, S., Pfeffer, K. & Bleul, C. C. Thymic medullary epithelial cell differentiation, thymocyte emigration, and the control of autoimmunity require lympho-epithelial cross talk via LTβR. *J. Exp. Med.* **198**, 757–769 (2003).
58. Bonito, A. J. et al. Medullary thymic epithelial cell depletion leads to autoimmune hepatitis. *J. Clin. Invest.* **123**, 3510–3524 (2013).
59. Burkly, L. et al. Expression of RelB is required for the development of thymic medulla and dendritic cells. *Nature* **373**, 531–536 (1995).
60. Rossi, S. W. et al. RANK signals from CD4⁺3⁺ inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. *J. Exp. Med.* **204**, 1267–1272 (2007).
61. White, A. J. et al. Sequential phases in the development of Aire-expressing medullary thymic epithelial cells involve distinct cellular input. *Eur. J. Immunol.* **38**, 942–947 (2008).
62. Irla, M. et al. Autoantigen-specific interactions with CD4⁺ thymocytes control mature medullary thymic epithelial cell cellularity. *Immunity* **29**, 451–463 (2008).
63. Hikosaka, Y. et al. The cytokine RANKL produced by positively selected thymocytes fosters medullary thymic epithelial cells that express autoimmune regulator. *Immunity* **29**, 438–450 (2008).
64. Venanzi, E. S., Gray, D. H. D., Benoist, C. & Mathis, D. Lymphotoxin pathway and Aire influences on thymic medullary epithelial cells are unconnected. *J. Immunol.* **179**, 5693–5700 (2007).
65. Martins, V. C., Boehm, T. & Bleul, C. C. Ltβr signaling does not regulate Aire-dependent transcripts in medullary thymic epithelial cells. *J. Immunol.* **181**, 400–407 (2008).
66. Gray, D., Abramson, J., Benoist, C. & Mathis, D. Proliferative arrest and rapid turnover of thymic epithelial cells expressing Aire. *J. Exp. Med.* **204**, 2521–2528 (2007).
67. Michel, C. et al. Revisiting the road map of medullary thymic epithelial cell differentiation. *J. Immunol.* **199**, 3488–3503 (2017).
68. Metzger, T. C. et al. Lineage tracing and cell ablation identify a post-Aire-expressing thymic epithelial cell population. *Cell Rep.* **5**, 166–179 (2013).
69. Nishikawa, Y. et al. Temporal lineage tracing of Aire-expressing cells reveals a requirement for Aire in their maturation program. *J. Immunol.* **192**, 2585–2592 (2014).
70. Zhang, S. L. & Bhandoola, A. Trafficking to the thymus. *Curr. Top. Microbiol. Immunol.* **373**, 87–111 (2013).
71. Cosway, E. J. et al. Formation of the intrathymic dendritic cell pool requires CCL21-mediated recruitment of CCR7⁺ progenitors to the thymus. *J. Immunol.* **201**, 516–523 (2018).
72. Abramson, J. & Anderson, G. Thymic epithelial cells. *Annu. Rev. Immunol.* **35**, 85–118 (2017).
73. Klein, L., Klugmann, M., Nave, K.-A., Tuohy, V. K. & Kyewski, B. Shaping of the autoreactive T-cell repertoire by a splice variant of self protein expressed in thymic epithelial cells. *Nat. Med.* **6**, 56–61 (2000).
74. Anderson, M. S. et al. Projection of an immunological self shadow within the thymus by the aire protein. *Science* **298**, 1395–1401 (2002). **This study is the first to uncover the functional role of AIRE in promiscuous gene expression and central tolerance induction.**
75. DeVoss, J. et al. Spontaneous autoimmunity prevented by thymic expression of a single self-antigen. *J. Exp. Med.* **203**, 2727–2735 (2006).
76. Fan, Y. et al. Thymus-specific deletion of insulin induces autoimmune diabetes. *EMBO J.* **28**, 2812–2824 (2009).
77. Gavanescu, I., Kessler, B., Ploegh, H., Benoist, C. & Mathis, D. Loss of Aire-dependent thymic expression of a peripheral tissue antigen renders it a target of autoimmunity. *Proc. Natl Acad. Sci. USA* **104**, 4583–4587 (2007).
78. Lv, H. et al. Impaired thymic tolerance to α-myosin directs autoimmunity to the heart in mice and humans. *J. Clin. Invest.* **121**, 1561–1573 (2011).
79. Sansom, S. N. et al. 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**, 1918–1931 (2014).
80. Meredith, M., Zemmour, D., Mathis, D. & Benoist, C. Aire controls gene expression in the thymic epithelium with ordered stochasticity. *Nat. Immunol.* **16**, 942–949 (2015).
81. Danan-Gotthold, M., Guyon, C., Giraud, M., Levanon, E. Y. & Abramson, J. Extensive RNA editing and splicing increase immune self-representation diversity in medullary thymic epithelial cells. *Genome Biol.* **17**, 219 (2016).
82. Derbinski, J., Schulte, A., Kyewski, B. & Klein, L. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. *Nat. Immunol.* **2**, 1032–1039 (2001). **This comprehensive study is the first to identify the phenomenon of promiscuous gene expression as an integral functional property of mTECs.**
83. Gäbler, J., Arnold, J. & Kyewski, B. Promiscuous gene expression and the developmental dynamics of medullary thymic epithelial cells. *Eur. J. Immunol.* **37**, 3363–3372 (2007).
84. Sousa Cardoso, R. et al. Onset of promiscuous gene expression in murine fetal thymus organ culture. *Immunology* **119**, 369–375 (2006).
85. Tykocinski, L.-O. et al. Epigenetic regulation of promiscuous gene expression in thymic medullary epithelial cells. *Proc. Natl Acad. Sci. USA* **107**, 19426–19431 (2010).
86. Kernfeld, E. M. et al. A single-cell transcriptomic atlas of thymus organogenesis resolves cell types and developmental maturation. *Immunity* **48**, 1258–1270 (2018).
87. Smith, K. M., Olson, D. C., Hirose, R. & Hanahan, D. Pancreatic gene expression in rare cells of thymic medulla: evidence for functional contribution to T cell tolerance. *Int. Immunol.* **9**, 1355–1365 (1997).
88. Avichezer, D. et al. An immunologically privileged retinal antigen elicits tolerance: major role for central selection mechanisms. *J. Exp. Med.* **198**, 1665–1676 (2003).
89. Gillard, G. O. & Farr, A. G. Features of medullary thymic epithelium implicate postnatal development in maintaining epithelial heterogeneity and tissue-restricted antigen expression. *J. Immunol.* **176**, 5815–5824 (2006).
90. Derbinski, J., Pinto, S., Rösch, S., Hexel, K. & Kyewski, B. Promiscuous gene expression patterns in single medullary thymic epithelial cells argue for a stochastic mechanism. *Proc. Natl Acad. Sci. USA* **105**, 657–662 (2008).
91. Villaseñor, J., Besse, W., Benoist, C. & Mathis, D. Ectopic expression of peripheral-tissue antigens in the thymic epithelium: probabilistic, monoallelic, misinitiated. *Proc. Natl Acad. Sci. USA* **105**, 15854–15859 (2008).
92. Cloosen, S. et al. Expression of tumor-associated differentiation antigens, MUC1 glycoforms and CEA, in human thymic epithelial cells: implications for self-tolerance and tumor therapy. *Cancer Res.* **67**, 3919–3926 (2007).
93. Pinto, S. et al. Overlapping gene coexpression patterns in human medullary thymic epithelial cells generate self-antigen diversity. *Proc. Natl Acad. Sci. USA* **110**, E3497–E3505 (2013).
94. Brennecke, P. et al. Single-cell transcriptome analysis reveals coordinated ectopic gene-expression patterns in medullary thymic epithelial cells. *Nat. Immunol.* **16**, 933–941 (2015). **Together with Sansom et al. (2014) and Meredith et al. (2015), this study is the first to use scRNA-seq analysis to address the complexity of promiscuous gene expression in mTECs at a single-cell level.**
95. Takase, H. et al. Thymic expression of peripheral tissue antigens in humans: a remarkable variability among individuals. *Int. Immunol.* **17**, 1131–1140 (2005).
96. Takaba, H. et al. Fezf2 orchestrates a thymic program of self-antigen expression for immune tolerance. *Cell* **163**, 975–987 (2015).
97. Nagamine, K. et al. Positional cloning of the APECED gene. *Nat. Genet.* **17**, 393–398 (1997).
98. Aaltonen, J. et al. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat. Genet.* **17**, 399–403 (1997).
99. Perniola, R. Twenty years of AIRE. *Front. Immunol.* **9**, 98 (2018).
100. Nishikawa, Y. et al. Biphasic Aire expression in early embryos and in medullary thymic epithelial cells before end-stage terminal differentiation. *J. Exp. Med.* **207**, 963–971 (2010).
101. Nuber, U. A., Schäfer, S., Stehr, S., Rackwitz, H. R. & Franke, W. W. Patterns of desmocollin synthesis in human epithelia: immunolocalization of desmocollins 1 and 3 in special epithelia and in cultured cells. *Eur. J. Cell Biol.* **71**, 1–13 (1996).
102. Hale, L. P. & Markert, M. L. Corticosteroids regulate epithelial cell differentiation and Hassall body formation in the human thymus. *J. Immunol.* **172**, 617–624 (2004).
103. Farr, A. G. & Anderson, S. K. Epithelial heterogeneity in the murine thymus: fucose-specific lectins bind medullary epithelial cells. *J. Immunol.* **134**, 2971–2977 (1985).
104. Perry, J. S. A. et al. Distinct contributions of aire and antigen-presenting-cell subsets to the generation of self-tolerance in the thymus. *Immunity* **41**, 414–426 (2014).
105. Lei, Y. et al. Aire-dependent production of XCL1 mediates medullary accumulation of thymic dendritic cells and contributes to regulatory T cell development. *J. Exp. Med.* **208**, 383–394 (2011). **Together with Perry et al. (2014), this pioneering study provides key mechanistic insights into the complementary roles of mTECs and DCs in antigen presentation in the thymus.**
106. Leventhal, D. S. et al. Dendritic cells coordinate the development and homeostasis of organ-specific regulatory T cells. *Immunity* **44**, 847–859 (2016).
107. Watanabe, N. et al. Hassall's corpuscles instruct dendritic cells to induce CD4⁺CD25⁺ regulatory T cells in human thymus. *Nature* **436**, 1181–1185 (2005).
108. Odaka, C. et al. TGF-β type II receptor expression in thymic epithelial cells inhibits the development of Hassall's corpuscles in mice. *Int. Immunol.* **25**, 635–642 (2013).
109. Mikušová, R., Mešťanová, V., Polák, Š. & Varga, I. What do we know about the structure of human thymic Hassall's corpuscles? A histochemical, immunohistochemical, and electron microscopic study. *Ann. Anat.* **211**, 140–148 (2017).
110. Banerjee, A., McKinley, E. T., von Moltke, J., Coffey, R. J. & Lau, K. S. Interpreting heterogeneity in interstitial tuft cell structure and function. *J. Clin. Invest.* **128**, 1711–1719 (2018).
111. Gerbe, F., Legraverend, C. & Jay, P. The intestinal epithelium tuft cells: specification and function. *Cell. Mol. Life Sci.* **69**, 2907–2917 (2012).
112. Farr, A. G. & Rudensky, A. Medullary thymic epithelium: a mosaic of epithelial 'self'? *J. Exp. Med.* **188**, 1–4 (1998).
113. Van Ewijk, W. Cell surface topography of thymic microenvironments. *Lab. Invest.* **59**, 579–590 (1988).
114. Gerbe, F. et al. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature* **529**, 226–230 (2016).
115. Yamaguchi, T. et al. Skn-1a/Pou2f3 is required for the generation of Trpm5-expressing microvillous cells in the mouse main olfactory epithelium. *BMC Neurosci.* **15**, 13 (2014).
116. Ohmoto, M. et al. Pou2f3/Skn-1a is necessary for the generation or differentiation of solitary chemosensory cells in the anterior nasal cavity. *Biosci. Biotechnol. Biochem.* **77**, 2154–2156 (2013).
117. Saqui-Salces, M. et al. Gastric tuft cells express DLK1 and are expanded in hyperplasia. *Histochem. Cell Biol.* **136**, 191–204 (2011).
118. Gerbe, F. et al. Distinct ATOH1 and Neurog3 requirements define tuft cells as a new sensory cell type in the intestinal epithelium. *J. Cell Biol.* **192**, 767–780 (2011).
119. Bjerknes, M. et al. Origin of the brush cell lineage in the mouse intestinal epithelium. *Dev. Biol.* **362**, 194–218 (2012).
120. Sato, A., Hamano, M. & Miyoshi, S. Increasing frequency of occurrence of tuft cells in the main excretory duct during postnatal development of the rat submandibular gland. *Anat. Rec.* **252**, 276–280 (1998).
121. Höfer, D. & Drenckhahn, D. Cytoskeletal markers allowing discrimination between brush cells and other epithelial cells of the gut including enteroendocrine cells. *Histochem. Cell Biol.* **105**, 405–412 (1996).

122. Kasper, M. et al. Colocalization of cytokeratin 18 and villin in type III alveolar cells (brush cells) of the rat lung. *Histochemistry* **101**, 57–62 (1994).
123. O'Leary, C. E., Schneider, C. & Locksley, R. M. Tuft cells — systemically dispersed sensory epithelia integrating immune and neural circuitry. *Annu. Rev. Immunol.* **37**, 47–72 (2019).
124. Nadsjombati, M. S. et al. Detection of succinate by intestinal tuft cells triggers a type 2 innate immune circuit. *Immunity* **49**, 33–41 (2018).
125. Von Moltke, J., Ji, M., Liang, H. E. & Locksley, R. M. Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature* **529**, 221–225 (2016).
126. Howitt, M. R. et al. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* **351**, 1329–1333 (2016).
127. Lei, W. et al. Activation of intestinal tuft cell-expressed *Sucnr1* triggers type 2 immunity in the mouse small intestine. *Proc. Natl Acad. Sci. USA* **115**, 5552–5557 (2018).
128. Schneider, C. et al. A metabolite-triggered tuft cell-ILC2 circuit drives small intestinal remodeling. *Cell* **174**, 271–284 (2018).

Acknowledgements

Research in the Abramson laboratory is kindly supported by the European Research Council (ERC-2016-CoG-724821), Israel Science Foundation (1796/16), Chan Zuckerberg Initiative, Sy Syms Foundation, Wohl Biology Endowment Fund, Erica Drake Fund, Slomo and Cindy Silvan Foundation, The Enoch Foundation, Ruth and Samuel David Gameraff Family Foundation and Lilly Fulop Fund for Multiple Sclerosis Research.

Author contribution

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.

Peer review information

Nature Reviews Immunology thanks A. Chidgey, Y. Takahama and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© Springer Nature Limited 2019