HHS Public Access

Author manuscript

J Immunol. Author manuscript; available in PMC 2023 October 01.

Published in final edited form as:

J Immunol. 2023 April 01; 210(7): 888–894. doi:10.4049/jimmunol.2200610.

Investigating Thymic Epithelial Cell Diversity Using Systems Biology Approaches

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Abstract

The thymus is an intricate organ consisting of a diverse population of thymic epithelial cells (TEC). Cortical and medullary TECs (cTECs and mTECs) and their subpopulations have distinct roles in coordinating the development and selection of functionally competent and self-tolerant T cells. Recent advances made in technologies such as single-cell RNA sequencing (scRNA-seq) have made it possible to investigate and resolve the heterogeneity in TECs. These findings have provided further understanding of the molecular mechanisms regulating TEC function and expression of tissue restricted antigens (TSAs). In this brief review, we focus on the newly characterized subsets of TECs and their diversity in relation to their functions in supporting T cell development. We also discuss recent discoveries on expression of self-antigens in the context of TEC development as well as the cellular and molecular changes occurring during embryonic development to thymic involution.

Introduction

The thymus is the primary organ responsible for the generation and maturation of T cells with antigen specificity resulting in a diverse repertoire of T cell receptors (TCRs). T cells that are unable to properly distinguish between self- and non-self-proteins risk promoting autoimmune disease. During their intra-thymic development, thymocytes are subjected to a complex selection process mediated by recognition and binding strength of their TCRs to self-peptide/MHC complexes presented by thymic epithelial cells (TEC). Cortical or cTECs are found in the cortex region of the thymus and mediate positive selection of TCRs capable of recognizing peptide/MHC complex. Following positive selection, cTECs and subsequently medullary TECs (mTECs) remove potentially autoreactive T cells that have high-affinity TCRs for self-antigens in a process termed negative selection (1). T cells with intermediate affinity are additionally redirected to a regulatory T-cell fate (2, 3). These mechanisms of deletion and diversion ensure that only thymocytes with low self-affinity will differentiate into effector T cells (T_{eff}) and establish central tolerance. As the role of the thymus is to “educate” developing T cells against self and non-self, TECs express up to 19,293 protein-coding genes or 88% of all protein-coding genes (4, 5), which is the highest number of genes known to be expressed in any cell type. Many of these genes encode for self-proteins normally only found in differentiated cell types and thus are called tissue-restricted antigens (TRAs). At the population level, TECs express almost all protein-coding genes with only individual mature mTECS expressing 1–3% of TRAs at any given time (4–8). Additionally, mTECs have a high turnover rate (9) and several

defined developmental states with varying gene expression patterns, all leading to a highly heterogeneous population.

With the ability to sequence heterogenous populations at single-cell resolution using single cell RNA sequencing (scRNA-seq) technology, we are now able to provide new insights into the functions of thymic epithelial cells and their development. While there have also been many studies done utilizing scRNA-seq on developing T cells (10), this review aims to focus on the current field of thymic epithelial cell function along with the emerging knowledge recently revealed by systems immunology approaches. Better understanding of the mechanisms in which thymic epithelial cell development and self-antigen expression is regulated can lead to insights as to why individuals have different susceptibilities to autoimmune deviations and provide better strategies for therapies involving thymic culture systems to generate T cells.

TEC heterogeneity & development

Generally, cTECs are defined as Epcam^{+}LY51^{+}CD45^{-} by flow cytometry and keratin 8 (KRT8) expression whereas mTECs are defined as Epcam^{+}UEA-1^{+}CD45^{-} and keratin 5 (KRT5). Early studies showed that mTECs had heterogeneous expression of MHC II and thus mTECs can be broadly divided into CD80^{lo}MHCII^{lo} (or mTEC^{lo}) and CD80^{hi}MHCII^{hi} (or mTEC^{hi}). It was hypothesized that MHCII^{hi} mTECs were major players in thymic tolerance induction and MHC II genes are the prevailing contributors of genetic susceptibility to autoimmune diseases such as Type 1 Diabetes (T1D), multiple sclerosis, and rheumatoid arthritis among others (11). The mTEC^{hi} population consists of cells that have high expression of *Aire* and *Fezf2* and early studies showed embryonic mTEC^{lo} cells gave rise to mTEC^{hi} in a reaggregate thymic organ culture (RTOC, (12). It is now appreciated that the mTEC^{lo} population includes both immature mTEC precursors as well as terminally differentiated mTECs that have previously expressed Aire (13, 14).

Recent studies using scRNA-seq and fate-mapping analyses indicate that mTEC heterogeneity, is more complex than previously thought and mTECs have recently been classified into four major subsets, termed mTEC I-IV (14, Figure 1). Table 1 resolves some of the naming variations among publications from different groups which we will refer to in this review. mTEC I is characterized by high expression of *Itag6*, *Ly6a* (15), *Pdpn* (16, 17) and *Ccl21* (18). This population is found at the cortical-medullary junction of the thymus and production of CCL21 regulates the migration of positively selected thymocytes. The mTEC II population consists of the previously named mTEC^{hi} group expressing *Aire, Fezf2, CD40, H2-Aa or CD74* and are the precursors to terminally differentiated mTEC III cells that express *Pigr, Ly6d, Spink5, Ivl, and Krt10* (15) but have lost their *Aire* expression (18, 19). Notably, mTEC IV cells resemble tuft cells that have been described at mucosal sites, including gut and lung epithelial. Tuft cells are a type of chemosensory epithelial cell most studied for their role in controlling helminth infections by initiating type 2 mucosal immunity. When comparing transcriptional signatures of both tuft cells in the intestinal epithelium and thymus they were found to be similar and express *Lrmp, Avil, Trpm5, Dclk1, Gng13, Llcam and Sox9*. Thymic tuft cells are also characterized by high expression of interleukin-25 (IL-25) and are critical for the development of IL-4 producing type 2 invariant

natural killer T (NKT2) in the thymus (19). Increased frequencies of ILC2 were also present in the thymus of *Pou2f3*$^{-/-}$ mice lacking tuft cells, however it is unknown whether this is linked to absence of IL-25 (15).

While it was previously thought that mTEC I gives rise to the fully differentiated mTEC II population (16, 17), a more recent investigation utilizing scRNA-seq technology found a cluster of cells they termed TAC-TECs (18) as the precursor to both the *Ccl21* producing mTEC I and Aire positive mTEC II populations (Figure 1). This was demonstrated using both Aire-lineage tracing and RANK-ligand transient ablation models to measure population kinetics. While TAC-TECs seem to be a precursor population, these cells did not express previously described canonical stem cell markers and were actively cycling, suggesting that they are an undifferentiated population in transition between stem cells and differentiated cells known as transit-amplifying cells (20, 21). When comparing the TAC-TEC gene signature to those of published transit-amplifying populations (21) they found a significant enrichment of previously described transit-amplifying genes (18).

Supporting this hypothesis, Dhalla et al also identified a proliferating mTEC cluster that could act as a bipotent mTEC progenitor that differentiated into both Aire+ mTEC II and CCL21+ mTEC I lineages (22). Cells in the proliferating cluster upregulated genes involved in proliferation such as *Mik67*, and expressed *Aire*, suggesting it could represent the proliferating mTEC^{hi} population previously reported (22–24). Analysis of the same data using RNA velocity, a different trajectory method that relies on pre- and post-spliced RNA reads, produced conflicting results that indicated rather than differentiating into CCL21+ mTEC I, the proliferating mTEC cluster seemed to be derived from the mTEC I population (25). Another study conducted scRNA-seq of mouse TECs throughout the 1st year of year of life also identified a cluster equivalent to proliferating TAC-TECs. However, their diffusion psuedotime analysis suggested that the CCL21+ mTEC I could bifurcate into the two mTEC trajectories progressing towards Aire+ mTEC II and the proliferating mTEC cluster (26) (Figure 1).

While mTECs control thymocyte selection, CD4+ thymocytes also control the cellularity of Aire-positive mTECs by activating RANK and CD40 to induce the NF-κB signaling pathway. This reciprocal signaling between thymocytes and mTECs is referred to as thymic crosstalk. In a recent study using high through-put RNA-sequencing, Lopes et al show that self-reactive CD4+ thymocytes induce in CD80^{lo} mTECs pivotal transcriptional regulators to control the composition, including precursors of mTEC II (Aire+), mTEC III (post-Aire cells), and mTEC IV (tuft-like mTECs) (27). They also showed upregulation in expression of TRAs, chemokines, cytokines, and adhesion molecules involved in T-cell development suggesting that self-reactive thymocytes are inducers of T-cell tolerance by controlling the developmental transcriptional programs of mTEC subsets (27).

Self-antigen expression regulation

TECs express many self-proteins normally found only in differentiated cell types and thus these genes are called tissue-restricted antigens (TRAs). While peripheral tissues have tight spatio-temporal control of gene expression during various stages of development, the diverse

expression of tissue specific genes observed in the thymus is referred to as promiscuous gene expression (PGE) (4, 6, 28). In mTECs, the transcription factor Autoimmune Regulator (Aire) positively regulates 3980 TRAs whereas Aire-independent genes consist of around 3947 TRAs. Of the AIRE regulated genes, approximately 533 are directly dependent on AIRE for their expression (Aire-dependent), while expression of the remaining genes is enhanced in the presence of AIRE (Aire-enhanced) (4). Aire-dependent TRAs as generally characterized by an enrichment of H3K27me3 histone mark (trimethylation of lysine-27 of histone H3) indicating a repressive chromatin state (4). Furthermore, loss-of-function mutations in *Aire* causes the autoimmune polyglandular syndrome type-1 (APS-1) which is marked by thymic export of self-reactive T_{eff} cells (29). Many of these tissue specific genes are known targets of Aire and the syndrome is characterized by severe organ-specific autoimmunity affecting parathyroid chief cells, steroidogenic cells of the adrenal cortex, pancreatic β-cells, gastic parietal cells, skin melanocytes, hepatocytes, gonads, and the lung. Recently, *Fezf2* was identified as a transcription factor capable of inducing TRA expression independently of *Aire* (30), suggesting other potential factors are likely to contribute to the regulation of TRAs in mTECs.

Despite expression of almost all protein-coding genes at the population level, TRA expression at single-cell resolution is heterogeneous with individual mature mTECs only expressing 1–3% of TRAs at a time (4–8). On average, in a single cell TRAs accounted for approximately 10% of all genes expressed (17) and TRA repertoires are not enriched for any particular peripheral tissue (4, 5, 8, 17). While Aire-dependent genes have a smaller frequency of genes expressed and have higher mean expression levels (4, 5, 8, 17), Fezf2-induced TRAs have the same frequency as non-TRA expression but similar mean expression levels when compared to Aire-dependent genes (17). Additionally, *Aire* expression is only found in the mTEC II populations while interestingly *Fezf2* was detected in both $mTEC^{lo}$ and $mTEC^{hi}$ populations (12, 18). While Aire expression is dependent on RANK signaling, both lymphotoxin β receptor (LTβR) and RANK signaling have been implicated in Fezf2 expression (30, 31). Wells et al reported conflicting results showing that Fezf2 expression was generally independent of RANK (18), therefore which signals induces Fezf2 expression needs further study. While Aire-KO and Fezf2-KO mice both develop autoimmunity in several peripheral target organs and show defective clonal deletion of autoreactive thymocytes (30, 32), the expression of exact target genes controlled by *Aire* and *Fezf2* has also remained incompletely defined.

Accumulating evidence using scRNA-seq to investigate regulation of self-antigen co-expression points to PGE expression being coordinated by mTEC developmental stages (17, 22, 33). For example, Derbinski et al, compared expression of a small set of self-antigens between $CD80^{lo}$ and $CD80^{hi}$ mTECs and found that the $CD80^{hi}$ population had upregulated expression of both Aire-dependent and Aire-independent TRAs (28). More recently, several groups performed scRNA-seq and found mTECs tend to have higher expression of TRAs as they mature suggesting that the heterogeneity of self-antigen expression tend to reflect mTEC maturation trajectory (17, 22, 33). Dhalla et al went further to sort and compare total “unselected” mTECs in addition to mTECs expressing specific TRAs, namely Tspan8 and GP2 protein, and found the pre-selected self-antigens tended to cluster within mTEC subpopulations (22). This was also reproducible when comparing different mouse strains

strongly suggesting that PGE in mTECs is regulated under an ordered and not random process (22). Another study also utilizing scRNA-seq of mTECs from Aire-KO mice found altered heterogeneity of mTECs and aberrant expression of CTLA-4 which was not found in wildtype (WT) mice (34). The ectopically expressed CTLA-4 was found to remove CD80/CD86 ligands expressed on thymic dendritic cells which attenuated their ability to provide co-stimulatory signals and present self-antigens transferred from mTECs, leading to impaired Treg production and autoimmunity in Aire-KO mice (34). Overall, it appears that mTECs shift through heterogenous patterns of promiscuous gene expression throughout development to eventually cover all protein-coding self-antigens.

Investigation of TRA expression by mTEC subset show very few TRAs are differentially expressed in the mTEC I population (17) and mTEC II expresses significantly elevated levels of Aire-enhanced and Aire-dependent genes. While mTEC III had been regarded as a passive step towards mTEC death, this population has been found to express the highest number of TRAs, including Aire-unaffected TRAs and similar numbers of Aire-enhanced and Aire-dependent TRAs compared to mTEC II (17). Similarly, Fezf2-dependent TRA expression also increases as the mTEC II population undergos maturation to mTEC III (17). Supporting this finding, Wells et al also show that TRA expression peaks well after initiation of Aire expression and was maintained even after Aire expression decreased (18). Overall, Miragaia and Wells findings both suggest that while Aire is critical for inducing TRA expression, it is not necessary for maintaining it (17, 18). Interestingly, Miragaia et al observed little divergence of the TRA repertoires between each stage of maturation (17). This suggests that the TRAs encountered by thymocytes anywhere in the medulla does not depend largely on the maturation stage of the surrounding mTECs. Specifically, mTEC I are found in the cortex-medulla junction, mTEC II at the periphery of the medulla and mTEC III towards the center of the medulla (14). However, these observations are based on mRNA quantities which does not correlate linearly with protein quantities. A recent study using trans-omics analysis, combining transcriptomic and proteomic analysis, further identified signature molecules at both the mRNA and protein level that functionally and developmentally characterize cTECs and mTECs (35). Proteomic profiling of cTECs and mTECs needs further investigation and will be useful as an unbaised and powerful tool to gain insight into the distinct machinery of protein processing and peptide presentation to shape the self-tolerant TCR repertoire in T cells.

Recent evidence is now suggesting that AIRE regulates TRA expression by first directly regulating mTEC differentiation which subsequently regulates TRA expression. This was recently shown using scRNA-seq of mTECs from Aire-augmented mice, where Nishijima et al found Aire-augmented and Aire-KO mice both had altered mTEC heterogeneity and that many of the same TRAs were downregulated in both groups of mice (33). One hypothesis for this finding is that given the expression of TRAs is dependent on mTEC maturation status, perturbations of this process in either Aire-KO or Aire-augmented mice might account for the downregulation of TRAs. However, the authors did suggest that there was a possibility that feedback mechanisms initiated by Aire overexpression may also be playing a role in transcriptional suppression of TRAs.

Overall, identification of direct Aire targets has been hampered by low and promiscuous expression of TRAs. Using ATAC-seq, ChIP-seq and a reporter gene assay, the authors were able to identify CCl25 as a canonical target downstream of Aire (33). While Ccl25-deficient mice showed some inflammatory changes in the salivary gland and kidney, they did not show obvious signs of autoimmunity. Ccl25 also did not affect expression of TRAs and differentiation programs in mTECs so how reduced Ccl25 develops organ-specific autoimmune disease awaits further study. In another study using transgenic mice, expression of model TRAs were directed under either the C-reactive protein (*CRP*) locus, whose Aire-independent expression is preferentially detected in the mTEC I subset, or the Insulin2 (*Ins2*) locus to restrict expression to Aire+ mTEC II subsets. Using these models of antigen expression, it was found that mTEC I supported TCR$\alpha\beta^{+}$ CD8$\alpha\alpha$ intraepithelial lymphocyte development whereas mTEC II restricted antigen preferentially induces Treg differentiation to impact control of infections agents and tumor growth (36).

About a decade ago, Aire-regulated genes were shown to colocalize in chromosomal clusters (37, 38). Since then, it has been accepted that AIRE's ability to recruit transcription factors to regions of closed chromatin would induce remodeling thus facilitating the co-expression of neighboring genes (29). The discovery that AIRE binds super enhancers supports this idea (39), providing a model that that explains both intra- and inter-chromosomal co-expression patterns of Aire regulated TRAs (8). Maragaia et al further investigated the gene clustering effect at the single cell level to determine how it changes in mTEC subsets and how it affects both Aire-enhanced and Aire-dependent TRAs (17). Overall, they found that genomic clustering tendency preferentially affects a minority of Aire-dependent TRAs and this effect seems to be established only in mTEC II and III, while limited in the mTEC I population (17).

From embryo to thymic involution

During mouse embryogenesis, an early rudiment of the thymus is evident in mouse gestation by embryonic day (E) 10–11. As development progresses, hematopoietic progenitors migrate to the thymus triggering the organ to double in size daily until birth. This formation and subsequent TEC differentiation is critically dependent on the expression of the transcription factor, *Foxn1* (40–42) and mutations in the FOXN1 gene are characterized by T-cell immunodeficiency, congenital alopecia, and nail dystrophy (43). Bipotent progenitors of cTECs and mTECs arise as early as day 12.5 (44), however in an embryonic thymus, cTECs arise earlier whereas later in life mTECs are the predominant population in the adult thymus (45). mTEC I and mTEC II subpopulations become detectable at E18.5 and can be the most proliferative cells in the TECs. mTEC III can be found in the 4-week-old thymus and mTEC IV cells are present on neonatal day 6. Molecular features of TECs also differ between embryonic and adult thymus. For example, while in the adult thymus cTECs and mTECs can be separated based on their expression of keratin-5 and kertin-8 respectively (46, 47), in the early embryonic thymus a keratin 5- and keratin 8-positive TEC subset is the dominant population (48, 49) making analysis of TEC populations by this approach difficult.

Recent studies using scRNA-seq has helped provide new insights into the developmental pathway of the thymus both at embryonic stage and up until adulthood. One study

characterizing the developmental dynamics of the embryonic thymus both *in vivo* and *in vitro* provided a single-cell transcriptional framework for thymus organogenesis (50). The study sampled thymic lobes beginning at embryonic day (E)12.5 and each day until birth, and they found Aire-expressing cells as early as E13.5. They also confirmed that mTEC III and IV were only found in the adult thymus (51) and not at birth (50). Additionally, they found that expression of autoimmune-implicated genes may begin during embryogenesis. Overall, their findings showed cellular heterogeneity of TECs dynamically changes with progression of thymus organogenesis, providing useful markers for developmental trajectories. Furthermore, they sequenced short term *in vitro* fetal thymic organ cultures (FTOCs) and found their transcriptome was comparable to *in vivo* development of thymii at similar timepoints (50). This provides evidence that *in vitro* thymic organ cultures are a physiologically relevant model and may be useful for future studies dissecting perturbations to thymic organ or T cell development. As an example, they showed that exogenous retinoic acid did not change the overall landscape of TEC compartments (50).

After birth the thymus continues to expand in size, although at a reduced rate, until peaking in size at ~4 weeks of age. It is then maintained in the adult mouse until puberty, around 8 weeks, after which it begins to decline (23). While TECs display considerable proliferative potential (23), it remains unclear why thymic involution occurs. Thymic involution refers to the diminished TEC cellularity and turnover, disrupted thymic architecture, decreased thymic output and reduced T cell function that occurs in an age-dependent manner (52–54). Aging, infections, pregnancy, stress, and other processes can all cause thymic involution, or atrophy (55–57). Involution and reduced T cell output has been linked to age-related incidence of cancer, infection, and autoimmunity (58). While reduced levels of the transcription factor, *Foxn1* contributes to thymic involution (59), genetic manipulation of cell-cycle regulators such as cyclins and E2F transcription factors can maintain thymic mass in aged mice (60, 61).

Another group performing transcriptome sequencing of neonatal TECs after birth included skin epithelial cells for comparison. They found that cell cycle progression is differentially regulated in TECs and skin epithelial cells and that many positive regulators of cell division are repressed in TECs relative to skin epithelial cells which could be related to thymic involution (62). Also supporting this hypothesis, Ki et al, showed thymic involution is associated with downregulation of cell-cycle genes in the $CD80^{lo}$ mTEC subset including decreased E2F3 activity in cTEC and $CD80^{lo}$ mTEC cells (51). Another study investigating transcriptional diversity of TECs through development also found increased proliferation and ribosomal biogenesis in fetal TEC and displayed diminishing expression with age (63). In this study, they showed that genes controlling ribosomal biogenesis and cell cycling in fetal stages in TEC development were targets of Myc which also had an age-correlated decline in expression (63). Overall, this further established Myc as a possible therapeutic target in modulating thymic regeneration and function.

Human thymus

While the thymus has been extensively studied using animal models, human immunity cannot be understood without further investigation into human TEC heterogeneity and

development. The study of human fetal thymus organogenesis has been mainly limited to morphological descriptions (64). Thymus organogenesis initiates from the third pharyngeal pouches during week 6 of gestation (65). During week 7, thymic and parathyroird primordium contains undifferentiated bi-potent thymic epithelial progenitor cells (TEPCs) (64) and at week 8, the differentiation of TECs occurs. After week 12, the cortical and medullary epithelial regions are distinct and CD4+ and CD8+ single-positive T lymphocytes appear (65).

To further characterize the molecular profiles of TECs and their interactions with developing thymocytes using human samples, two groups took single-cell transcriptomic approaches. Park et al, sequenced thymii spanning from embryonic, fetal, pediatric, and adult stages (66) whereas Zeng et al, analyzed thymii from the thymus, AGM (aorta-gonad-mesonephros) region, liver and blood of human embryos and fetuses (67). Park et al, found TEC populations that were conserved from mouse to human included PSMB11-positive cTECs, KRT14-positive mTEC I, Aire-expressing mTEC II, and KRT1-expressing mTEC III. mTEC IV tuft-like cells were found and enriched but were not specifically expressing DCLK1 or POU2F3. Additionally, there were two populations specific to humans which included MYOD1- and MYOG- expressing myoid cells, TEC(myo)s and NEUROD1-, NEUROG1- and CHGA-expressing TEC(neuro)s, that resemble neuroendocrine cells (66).

Another study investigated the transcriptional landscape of early thymic epithelial cell development and potential cell-cell interactions during early thymus organogenesis by using cells from multiple hemogenic and hematopoietic sites spanning embryonic and fetal stages. In human thymic primordia, TECs are relatively rare, accounting for about 1% of total cells (67, 68). Similarly in mice, few mature mTECs were detected during the early embryonic stages evidenced in the absence of *Aire* (67, 69–71) whereas cTECs proliferated and developed at a significantly faster rate than mTECs. Using prediction methods to calculate cellular interactions (72) they found that interactions between TECs and mesenchymal cells, as well as those between TECs and endothelial cells were the strongest, suggesting that cross talk between each type of stromal cell was important for early thymus organogenesis (67). Additionally, both embryonic and fetal early thymic progenitors (ETPs) had intensive interactions with TECs (67), consistent with the role of TECs in the hematopoietic progenitor-seeding thymus and T cell development.

The study of human thymus development has been limited; however, a recent study was able to isolate and expand clonogenic CD49f expressing mTEC and cTECs which could repopulate whole-organ scaffolds to reconstitute an anatomic phenocopy of the human thymus. The authors showed that the repopulated thymus scaffolds were able to support mature T cell development *in vivo* after transplantation into humanized immuno-deficient mice (73). Additionally, scRNA-seq analysis of the expanded cells in culture provided gene signatures useful for dissecting the complexity and properties of these thymic clonogenic cells. Further development of this organ reconstruction system and other *ex vivo* tools (73–76) will be valuable to address the roles of TECs and other human stromal cells (e.g. dendritic cells) during both organogenesis and thymopoesis.

Conclusions

Unbiased transcriptomic analysis has been powerful in advancing our understanding of TECs. Global gene expression analysis identified promiscuous gene expression in mTECs (4, 6, 28, 77) and single-cell RNA-sequencing has revealed enormous diversity in mTEC subpopulations, including the novel tuft-like cell population (13, 15). Recent discoveries have contributed to a new appreciation of TEC diversity to provide multiple thymic microenvironments supporting different stages of thymocyte development. With new tools to study cell heterogeneity, we can further understand the mechanisms contributing to their development and identify new markers for each population. Overall, research in TEC function contributing to thymocyte development and their molecular characteristics during development will provide better understanding of the etiology of multiple T-cell-related diseases. These insights will improve strategies for T-cell based immunotherapies in cancer and autoimmune diseases.

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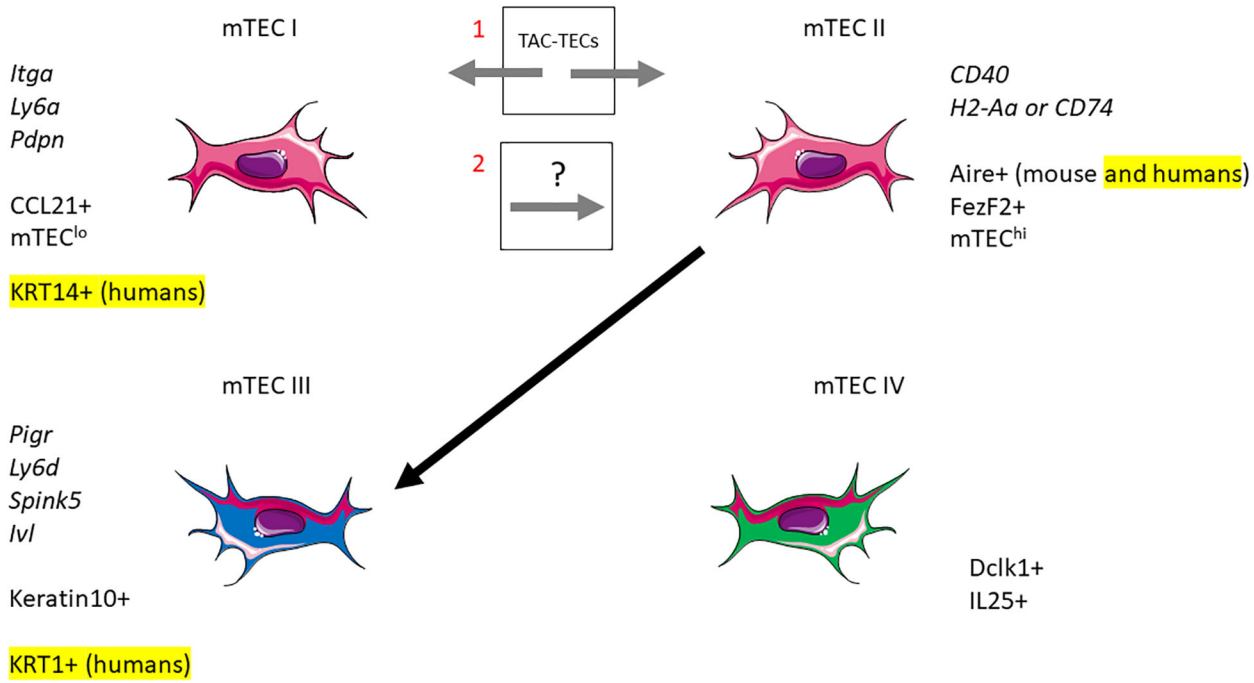

Figure 1. Phenotypic markers and pathways in mTEC development.

The mTEC I subset is part of the mTEC^{lo} compartment and characterized by CCL21 expression. mTEC II are the classically mature Aire+ mTEC^{hi} populations. mTEC III are the post-Aire terminally differentiated mTECs that also are found in the mTEC^{lo} compartment and mTEC IV are the newly found tuft-like cells. Additionally, there has been a newly characterized proliferating TAC-TEC subset and whether this subset is a progenitor of mTEC I and mTEC II (18), or mTEC I leads to the TAC-TEC and mTEC II populations (26) requires further study.

Table 1.

mTEC subsets.

Comparison of mTEC nomenclature used by different scRNA-seq studies

Bornstein (15)	**mTEC I**	**mTEC II**	**mTEC III**	**mTEC IV**	**Identified thymic tuft-cells**
Miragaia (17)	jTECs	mTEC^{hi}	mTEC^{lo}		jTECs precursors give rise to mTEC^{hi} → mTEC^{lo}
Wells (18)	*Ccl21*-high	Aire-positive	Late-Aire	Tuft	TAC-TEC population gives rise to both *Ccl21-high* and Aire-positive populations
Lebel (36)	mTEC^{lo}	mTEC^{hi}	Post-Aire mTEC	Tuft cells	Model antigen with biased mTEC^{hi} expression supports Treg differentiation
Lopes (27)	CCL21^{+}	Aire^{+}	Post-Aire	Tuft-like	Self-reactive CD4^{+} thymocytes regulate Aire^{+} precursors, post-Aire and tuft-like mTEC populations