Immunology Letters 215 (2019) 24–27

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Medullary thymic epithelial cells: Deciphering the functional diversity beyond promiscuous gene expression

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ARTICLE INFO

Keywords:
Thymus
Thymic epithelial cells
Medullary thymic epithelial cells

ABSTRACT

Within the thymus, cortical and medullary thymic epithelial cells (cTECs and mTECs, respectively) provide unique microenvironments for the development of T cells that are responsive to diverse foreign antigens while self-tolerant. Essential for tolerance induction, mTECs play a critical role in negative selection and T regulatory cell differentiation. In this article, we review the current knowledge on the functional diversity within mTECs and discuss how these novel subsets contribute to tolerance induction and are integrated in the complex blueprint of mTEC differentiation.

1. Introduction

T cells express a variety of T cell receptors (TCRs) that respond to a myriad of pathogen and cancer cell-derived antigens while being simultaneously tolerant to our own tissues. This diverse pattern of recognition depends on the random rearrangement of V(D)J gene segments of TCR during T cell development, which apart from of generating T cells with favourable specificities, can also produce autoreactive T cells [1]. Cortical and medullary thymic epithelial cells (cTECs and mTECs, respectively) are essential to impose the basic principles that control T cell immunity and self-tolerance [2]. While cTECs promote T cell lineage commitment and positive selection, mTECs regulate negative selection, T regulatory cell differentiation and the final steps of single positive thymocyte maturation [3]. Despite defining functionally distinct compartments [4], cTECs and mTECs arise from common bipotent TEC progenitors (TEPs) that exist in the embryonic and postnatal thymus [5,6]. TEC differentiation is strictly dependent on the forkhead box protein N1 (Foxn1) transcription factor (reviewed in detail in [7]).

The chief role of mTECs in tolerance induction depends on their capacity to express virtually all tissue restricted antigens (TRAs) [8]. This process anticipates the presentation of peripheral self-antigens to developing T cells, so that potentially autoreactive thymocytes are eliminated or deviated to the T regulatory cell lineage. Autoimmune regulator (Aire) and recently described forebrain embryonic zinc finger-like protein 2 (Fezf2) are two known regulators of promiscuous gene expression (PGE) in mTECs [9]. The advent of single-cell RNA analysis has revealed that individual mTECs express unique sets of TRA genes, so that the comprehensive representation of all genes in the genome is only achieved by multiple mTECs [10–12]. Apart from this heterogeneous expression pattern, recent findings highlight that mTECs are composed of functionally distinct subsets that might not be immediately linked to their promiscuous gene expression capacity. Here, we discuss how unravelling mTEC complexity could help decode the mechanisms that regulate their development and function in tolerance induction.

2. mTEC progenitors and their maturation requirements

Although it was initially considered that cTECs and mTECs synchronously differentiate from common bipotent progenitors, recent studies have provided evidence for the existence of TEPs that express cTEC traits (*e.g.* β5t, CD205, IL-7 and CCRL1) and have the potential to generate both cTECs and mTECs [13–16]. These observations led to a refined differentiation model whereby TEC progenitors transverse through the cTEC lineage prior to the commitment into mTECs [17] (Fig. 1). Still, the identity of bipotent TEPs or unipotent cTEC precursors at the single-cell level as well as the molecular blueprint that drives the differentiation of cTECs remains unknown. In contrast, the nature of mTEC-restricted precursors (mTEPs) and the molecular principles that drive the maturation and maintenance of mTECs are better understood [18]. In particular, initial reports identified

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https://doi.org/10.1016/j.imlet.2019.01.012
Received 4 January 2019; Received in revised form 28 January 2019; Accepted 30 January 2019
Available online 07 March 2019

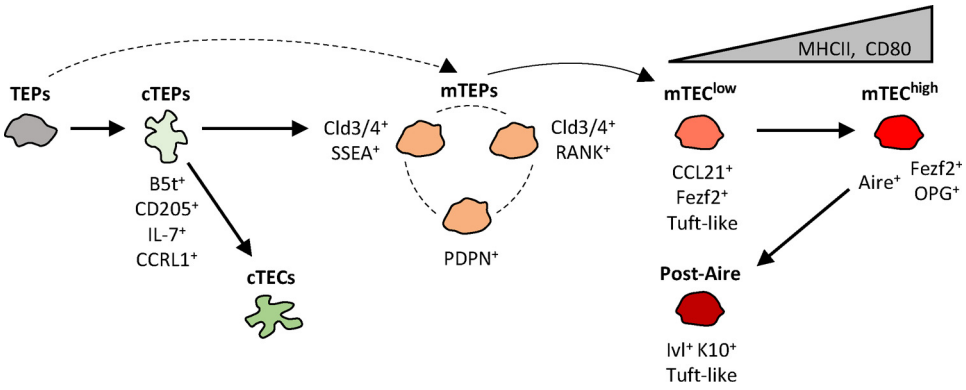

Fig. 1. Schematic representation of mTEC development and of the characterized phenotypic and functional subsets. Medullary thymic epithelial cells (mTECs) derive from bipotent thymic epithelial progenitors (TEPs). TEPs expressing cTEC-associated traits (cTEPs) participate in the differentiation of mTEC-committed progenitors (mTEPs). The highly heterogeneous mTEC compartment contains several subsets with specific roles in T cell development. mTECs can be broadly divided into mTEC^{low} and mTEC^{high}, based on the expression levels of MHCII and CD80, the latter also containing Aire-expressing cells. In turn, Aire-expressing cells can transition into a post-Aire stage, which is characterized by the downregulation of MHCII and CD80. Beyond the populations defined by MHCII and CD80 expression levels, mTECs possess a higher degree of phenotypic and functional diversity, defined by the expression of the listed cell surface or secreted phenotypic markers, chemokines, transcription factors or specific transcriptional programs.

Claudin3$^+$ and Claudin4$^+$ (Cld3/4$^+$) mTEPs in the early embryonic thymus that exclusively generate mTECs [19]. A subsequent study revealed that the expression of stage-specific embryonic antigen A (SSEA) further enriched for mTEPs within Cld3/4$^+$ cells [20], which derive from β5t-expressing cells [21]. More recently, Cld3/4$^+$SSEA$^-$RANK$^+$ mTEPs have also been defined to exist in the embryonic thymus [22]. In the post-natal thymus, Podoplanin (PDPN)$^+$ cells identify another mTEP population that resides at the cortico-medullary junction and substantially contributes to the maintenance of the adult mTEC compartment [23]. Despite these advances, the precursor-product relationship between these novel mTEC subsets is unclear (Fig. 1). In particular, future studies should address whether these subsets constitute sequential stages of the same differentiation program or separate lineages.

The development of mTECs is highly dependent on instructive signals provided by the hematopoietic cells within the thymus [24,25]. The thymocyte-TEC interactions, commonly termed “thymic crosstalk”, engage the canonical and noncanonical nuclear factor kappa B (NF-κB) signalling pathways in mTECs and their progenitors, which in turn activate the transcriptional programs required for their maturation [26]. As such, the disruption of genes in these pathways results in impaired medulla organization and autoimmunity [27–29]. Later studies coupled the induction of NF-κB signalling to the upstream engagement of specific members of the tumour necrosis factor receptor superfamily (TNFRSF), including receptor activator of NF-κB (RANK), CD40 and lymphotoxin β receptor (LTβR) [4]. In this regard, LTβR signalling has been implicated in the maintenance of the Cld3/4$^+$SSEA$^+$ mTEP population, particularly in the neonatal thymus [30], and in the induction of RANK expression [31]. Accordingly, Cld3/4$^+$SSEA$^+$ cells express LTβR [20] and emerge prior to the RelB-dependent Cld3/4$^+$SSEA$^-$RANK$^+$ mTEPs [22]. Additionally, the engagement of RANK signalling upregulates the expression of CD40 during mTEC development [32]. Collectively, these findings suggest a sequential requirement for activation of the TNFRSF members to implement the mTEC differentiation program.

3. Phenotypic and functional complexity of mTECs

Over the last years, several studies revealed a high degree of functional heterogeneity within mTECs, which correlates with the distinct thymopoeitic stages supported by these cells. Earlier reports uncovered a first level of mTEC diversity via the characterization of cells expressing low and high levels of MHCII and CD80, (mTEC^{low} and mTEC^{high}, respectively), which share a precursor-product relationship [33]. Subsequently, it was discovered that a fraction of mTEC^{high} contains Aire-expressing cells [34]. RANK signalling plays a dominant role in the development of Aire$^+$ mTECs, whereas CD40 also contributes to this process [35]. Although LTβR signalling was initially linked to the development of the Aire$^+$ lineage [36], subsequent studies uncoupled these two processes [37,38]. Instead, LTβR signalling was shown to be involved in the organization of the medullary compartment [39]. The characterization of RANK reporter mice revealed that RANK expression can be found in both the mTEC^{low} and mTEC^{high}, including Aire$^+$ cells, and therefore, it seems not to demark a discrete mTEC population [40]. Importantly, the intensity of RANK signalling is tightly controlled *in vivo*. In particular, Osteoprotegerin (OPG) is a decoy receptor for RANKL that inhibits RANK signalling in mTECs [41]. Interestingly, OPG is produced within the mTEC^{high} population, predominantly by Aire$^+$ cells [40], revealing a further degree of functional heterogeneity within the medullary microenvironment (Fig. 1).

Beyond its role in TRA expression, several reports suggested an alternative function for the Aire protein in the regulation of mTEC homeostasis [42]. Supporting this hypothesis, mTEC^{high} accumulate in Aire-deficient mice [33,43] and present an altered mTEC morphology [44,45]. One of the models to explain this phenotype argues for a pro-apoptotic effect of Aire, so that mTEC^{high} display an increased survival and higher numbers in its absence [33]. An alternative scenario has been constructed in which Aire expression is required for the completion of mTEC differentiation [46]. In this regard, Aire$^+$ cells differentiate into a post-Aire stage, which is typified by the downregulation of MHCII and CD80, reduced TRA expression and the expression of markers associated with terminal keratinocyte differentiation [47–49]. Although both models are not necessarily mutually exclusive, the identification of the post-Aire stage within the mTEC^{low} subset revealed a further degree of diversity within this compartment. More recently, it has been shown that mTEC^{low} also include CCL21-producing cells, which develop in a LTβR-dependent manner and possess an important role in the migration of positively selected thymocytes to the medulla [50]. As such, disruption of CCL21 expression leads to a defective localization of thymocytes to the medulla and perturbs tolerance induction [51].

The existence of Aire-independent TRA expression [52] implies that PGE is controlled in mTEC by other regulators. The transcription factor Fezf2 has been recently defined as a regulator of the expression of some Aire-independent TRAs [53]. Fezf2$^+$ cells are found both in mTEC^{low} and mTEC^{high} [53] and represent another example that highlights the functional heterogeneity of mTECs. Although the development of Fezf2$^+$ cells was initially described as LTβR-dependent [53], a subsequent study has linked its expression to RANK signalling [39]. Further studies are warranted to settle the role of this seemingly important population for tolerance induction. Overall, these findings highlight that mTEC^{low} represent a multifunctional population, containing not only mTEC^{high} precursors, but also terminally differentiated post-Aire cells and other functionally competent mTECs with specific roles in T cell development (Fig. 1).

The latest addition to the expanding catalogue of mTEC subsets has

been provided by two complementary studies. Taking advantage of unsupervised single-cell RNA-sequencing and fate-mapping analyses, both reports have identified a novel mTEC subpopulation that intriguingly resembles mucosal-associated epithelial tuft cells [54,55]. Tuft-like mTECs have a unique transcriptional program that differs from the remaining mTEC subsets and are characterized, among others, by the production of IL-25, a characteristic cytokine of mucosal tuft cells [54,55]. This population exist within both pre- and post-Aire $mTEC^{low}$ (Fig. 1), and their development is critically dependent on the transcription factor Pou2f3 [54]. Albeit with a normal T cell differentiation, mice deficient in tuft cells, via genetic ablation of Pou2f3, present specific alterations in type-2 cells. While Miller et al. show that $Pou2f3^{-/-}$ mice present a defective tolerance to tuft cell-associated antigens, including IL-25, and a reduction in NKT2 cells [54], Bornstein et al. demonstrate that these mutants have an increased frequency of thymic ILC2s [55]. The presence of this unique population in the thymus raises multiple questions on the basis of their development and function. It is unlikely that these cells result from a coincidental assembly of randomly expressed tuft-restricted TRAs. A more favourable possibility is that they arise from a programmed mTEC differentiation pathway. These findings raise the possibility that other specialized populations of epithelial cells from other tissues could be represented within the thymus, perhaps with additional effects in tolerance induction.

4. Concluding remarks

The distinction of self and non-self is an essential aspect of T cell immunobiology that is highly dependent on the selection processes governed by mTECs. Overall, the findings described in this review portray the mTEC compartment as an expanding universe of functionally distinct but perhaps interconnected subsets (Fig. 1), which warrant further exploration. Given the inherent complexity of this population, a comprehensive blueprint of the mTEC developmental program has not yet been defined. Regarding the origin of mTECs, we still lack a solid understanding of the mechanisms that regulate the maintenance of the different mTEP pools and their possible lineage relationship. Also, the molecular roadmap that leads to the establishment of the aforementioned different functional subsets remains elusive. In this regard, it has been proposed that all mTECs follow a differentiation program predestined to Aire expression [56]. Alternatively, $Aire^{+}$ cells might constitute only one of several distinct possible lineages. Future research will benefit from the establishment of novel stage-specific markers, which in combination with RNA-seq and fate-mapping mouse models, can further dissect new developmental stages in mTECs. These efforts can ultimately uncover the inner workings of this unique cell population and provide a steppingstone for the understanding of human mTEC biology, thus opening novel avenues for the development of immunotherapies.

Acknowledgements

The authors thank the members of the laboratory for their past and present contributions. They apologize for not referring to all primary literature owing to space limitations. This study was supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 637843-TEC_Pro) starting grant attributed to N.L.A., by FEDER (Fundo Europeu de Desenvolvimento Regional) funds through the Operational Competitiveness Programme–COMPETE, and by National Funds through Fundação para a Ciência e Tecnologia (FCT) under the project FCOMP-01-0124-FEDER-021075 (PTDC/SAU-IMU/117057/2010), by NORTE-01-0145- FEDER-000012–Structured program on bioengineered therapies for infectious diseases and tissue regeneration, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (FEDER); by FEDER funds through the COMPETE 2020–Operational Programme for Competitiveness and Internationalisation (POCI), Portugal 2020 and by Portuguese funds through FCT/Ministério da Ciência, Tecnologia e Inovação in the framework of the project Institute for Research and Innovation in Health Sciences (POCI-01-0145- FEDER-007274).

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