SCIENCE CHINA
Life Sciences

SPECIAL TOPIC: Impaired immune response and immunologic adjuvants
• REVIEW •

May 2013 Vol.56 No.5: 392–398
doi: 10.1007/s11427-013-4482-4

Medullary thymic epithelial cells, the indispensable player in central tolerance

SHI YaoYao[1,2] & ZHU MingZhao[1*]

[1]*Key Laboratory of Infection and Immunity, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China;*
[2]*University of Chinese Academy of Sciences, Beijing 100080, China*

Received March 8, 2013; accepted March 21, 2013

Crosstalk between thymocytes and thymic epithelial cells is critical for T cell development and the establishment of central tolerance. Medullary thymic epithelial cells (mTECs) play important roles in the late stage of T cell development, especially negative selection and Treg generation. The function of mTECs is highly dependent on their characteristic features such as ectopic expression of peripheral tissue restricted antigens (TRAs) and their master regulator—autoimmune regulator (Aire), expression of various chemokines and cytokines. In this review, we summarize the current understanding of cellular and molecular mechanisms of mTEC development and its functions in T cell development and the establishment of central tolerance. The open questions in this field are also discussed. Understanding the function and underlying mechanisms of mTECs will contribute to the better control of autoimmune diseases and the improvement of immune reconstitution during aging or after infection, chemotherapy or radiotherapy.

mTECs, T cell development, Treg, negative selection, autoimmunity

Citation: Shi Y Y, Zhu M Z. Medullary thymic epithelial cells, the indispensable player in central tolerance. Sci China Life Sci, 2013, 56: 392−398, doi: 10.1007/s11427-013-4482-4

Thymus is the indispensable organ for T cell development in mammals. Haematopoietic progenitors, originating from bone marrow, populate the thymus to go through positive selection and negative selection to become mature thymocytes before their emigration to periphery. Thymic epithelium is not only the essential organizer of thymus, but also the important educator for T cell development. Thymic epithelium not only directs positive selection of self-MHC restricted T cells for them to achieve immune competency [1], it is also critical for clonal deletion of self-reactive T cells to establish T cell central tolerance [2,3] (Figure 1).

Thymus medulla is considered the major site for negative selection. Defective development of medullary thymic epithelial cells (mTECs), as observed in *Relb*$^{-/-}$, *Ikka*$^{-/-}$, *Traf6*$^{-/-}$ and *aly*/*aly* mice, is often associated with autoimmune phenotype [4–7]. The last decade has seen a growing recognition of the role of mTECs in T cell development and central tolerance. In this article, we review the underlying cellular and molecular mechanisms of mTECs development and function, and discuss unresolved mysteries in this field.

1 Aire, the master controller for TRA ectopic expression in mTECs

An essential goal of thymic development is to ensure the selection of a T cell repertoire both immune competent and self-tolerant. For many years, immunologists wondered how mammals achieve immune tolerance to self-antigens that are restrictedly located in peripheral tissues. T cells that are

*Corresponding author (email: zhumz@ibp.ac.cn)

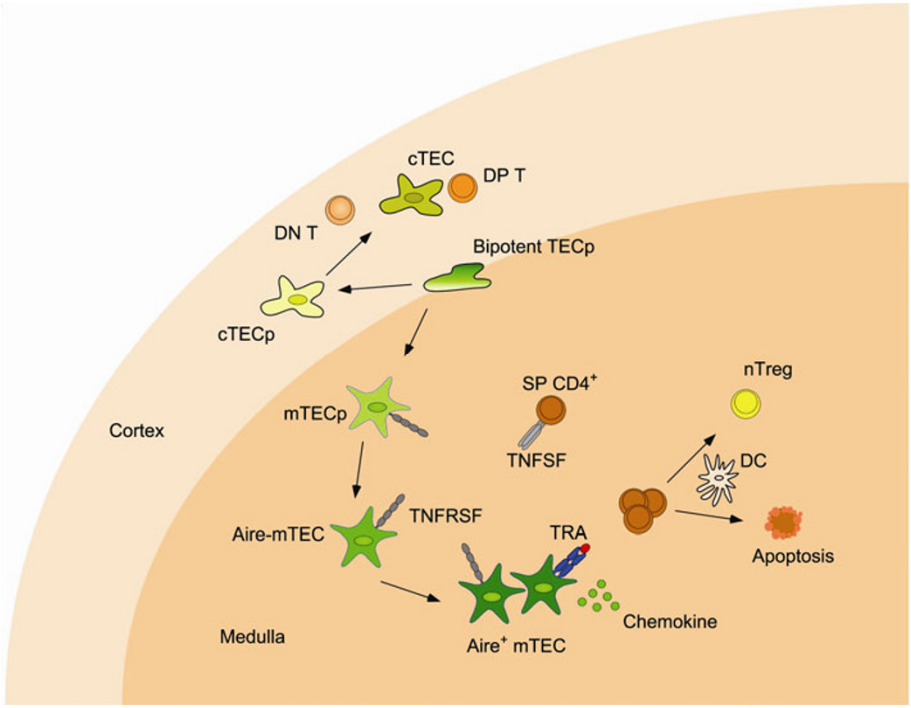

Figure 1 Development and function of medullary thymic epithelial cells. Both medullary and cortical thymic epithelial cells are considered derived from common bipotent progenitors. mTECs undergo a series of maturation stages acquiring unique properties of expressing self-antigens, chemokines, adhesion molecules and other co-stimulation molecules. mTECs directly present or bypass self-antigen to other antigen presenting cells, such as dendritic cells, to instruct negative selection and nTreg generation, two major mechanisms of central tolerance. Aire, autoimmune regulator; cTEC, cortical thymic epithelial cell; DN, double negative; DP, double positive; mTEC, medullary thymic epithelial cell; nTreg, natural regulatory T cell; SP, single positive; TECp, thymic epithelial cell progenitor; TNFSF, tumor necrosis factor superfamily; TNFRSF, tumor necrosis factor receptor superfamily; TRA, tissue restricted antigen.

reactive to ubiquitous self-antigens are deleted in the thymus, a process during which DCs are previously believed the most efficient scavenger to eliminate self-reactive thymocytes [8], and contribute to the removal of 50% positively selected thymocytes in negative selection [9,10]. However, how the same central mechanism might forestall autoimmunity against peripheral tissue-restricted antigens (TRAs) remained a perplexing mystery until recently. A liver-associated antigen expression in a minor fraction of mTECs was identified autonomously leading to deletion of specific $CD4^+$ T cells without the contribution of cross-presentation by haematopoietic antigen-presenting cells [11,12]. Careful study revealed that mTECs are a unique cell population in thymus expressing an abundant profile of TRAs suggesting a general mechanism of mTECs in inducing TRA-specific self-tolerance [13].

Given the increasing evidence implying the importance of ectopic TRA expression in mTECs for the establishment of self-tolerance, people started to wonder how those TRA expression is regulated. Following clues from a rare human autoimmune syndrome, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) [14,15], Mathis group discovered that autoimmune regulator (Aire) is a critical factor controlling a huge bunch of TRA expression in mTECs [16]. Aire deficient mice display autoantibodies against various peripheral organs. TRA-specific T cell development analysis in Aire deficient mice revealed a dramatic defect in clonal deletion of self-reactive T cells in the thymus [17].

It is still a mystery how a single molecule can regulate the expression of such a broad spectrum of TRAs in mTECs. Two models were proposed: progressive restriction model and terminal differentiation model [18]. In the former model, Aire expresses in immature TRA-expressing mTEC precursors, and directs mTEC differentiation into progressively restricted epithelial cell subsets, with individual cells taking on different fates. In the latter one, more diversified and abundant TRAs expression accompanies mTEC differentiation and maturation. Most research supports the terminal differentiation model. In fact, Aire is highly expressed in MHC $II^{high}CD80^{high}$ mature mTEC subset [19–21]. Moreover, $Aire^+$ mTECs are found a postmitotic, terminal population [22].

On the molecular level, Aire might control TRA expression through several mechanisms. (i) The plant homeodomain (PHD) finger of Aire preferentially binds unmethylated H3K4, a mark of relatively inactive chromatin, to initiate gene expression [23–25]. (ii) Aire could interact with transcriptional elongation factor TOP2a to initiate DNA double-stranded breaks (DSBs) [26]. These DSBs would acti-

vate DNA/PK/Ku80, PARP-1 and other proteins associated with Aire to attract chromatin remodeling complexes and thereby alter chromatin architecture [27]. (iii) Mutations in Caspase recruitment domain (CARD) and SAND (SP100, AIRE1, NucP41/P75 and DEAF1) domain of Aire decrease its transactivation ability [28,29]. (iv) AIRE in mTECs is capable of binding and recruiting the positive transcription elongation factor b (P-TEFβ) complex to RNA polymerase II to target gene promoters to promote transcriptional elongation [30]. (v) The most recent work demonstrates that Aire activates TRA transcription not through specific recognition of TRA gene promoters but by releasing stalled polymerases [31].

Although Aire remains the only one identified transcriptional factor regulating TRAs ectopic expression in mTECs since its discovery a decade ago, evidences exist for Aire-independent TRA regulation pathway. Such examples include type II collagen, casein β and glutamic acid decarboxylase 67 [32,33]. We have found the ectopic expression of type II collagen (CII) in mTECs is Aire-independent, but lymphotoxin (LT) dependent. The failure to express LT leads to severely reduced production of CII in mTECs, and thereby causes overt autoimmunity to CII. The expression level of CII in mTECs is normal in the absence of Aire. Boyd group also demonstrated that Aire-independent TRAs are expressed in both MHC II^{high} and MHC II^{low} mTEC subsets [33]. In addition, LT is critical for the normal expression of these Aire-independent TRAs within the Aire-null MHC II^{low} mTEC population [33]. It remains an interesting question how the ectopic expression of TRAs in mTECs is regulated by LT pathway independent of Aire.

2 Negative selection by ectopic TRA expression in mTECs

TCR signaling by peptide-MHC engagement is crucial for T cell development and fate determination. Ectopic expression of TRAs and their presentation in thymus significantly shape the T cell repertoire. One crucial progress the above ectopic TRAs engaged in is the induction of thymocyte negative selection.

TRAs expressed in mTECs can be both directly presented by mTECs and indirectly transferred to and presented by other thymic APCs to developing thymocytes for negative selection [34]. Earlier study showed that $CD8^+$ OT-I T cells can be efficiently negatively selected in RIP-mOVA thymus by both mTECs or haematopoietic APCs, likely DCs; while negative selection of $CD4^+$ OT-II T cells in this model can only be mediated by haematopoietic APCs [35]. This study suggests that the ectopic OVA expression in mTECs can be both directly presented by mTECs themselves or indirectly by thymic DCs after antigen transfer. Later work conclusively demonstrated that TRAs expression in mTECs can be indeed transferred to thymic DCs [36]. Interestingly, Aire seems also important for the intercellular antigen transfer, although the mechanism remains elusive [37].

The intercellular antigen transfer model reconcile the perplexity how a set of very few mTECs expressing given individual TRA can efficiently shape such a robust and self-tolerant thymocyte repertoire. Involvement of professional DCs might thus significantly increase the presentation and usage efficiency of limited TRAs. The intercellular antigen transfer is also well consistent with the facts that Aire-expressing mTECs are terminally differentiated cells with higher turnover rate than Aire-null mTECs and Aire promotes mTECs apoptosis [22]. Meanwhile, it is of note that other scenarios exist to increase TCR repertoire shaping by such little amount of TRAs. First, current assessment of TRA expression in mTECs may provide just a snapshot and thus could underestimate the amount of TRA expression in a temporal and spatial way. Second, the peptide-MHC complex is relatively stable and long-lasting in thymus, thus would allow continuous presentation by more APCs than those actually transcribe the TRA gene, considering the "antigen spreading" scenario [34,38]. Third, thymocytes scan medulla highly efficiently for peptide-MHC complex through serial short-lived interactions with thymic APCs in a similar way in peripheral lymph node during T cell priming thus increase the efficiency of encounterment of thymocytes with rare APCs presenting specific antigen [39]. Therefore, multiple mechanisms ensure mTECs express and present limited amount of TRAs efficiently to delete autoreactive T cells.

3 Treg repertoire shaped by mTECs?

Thymus is also the site for the development of natural $CD4^+Foxp3^+$ regulatory T cells (Treg). Although early study shows that most $Foxp3^+$ cells are located in the medulla of thymus suggesting an important role of mTECs in Treg development [40,41], the specific role of mTECs in Treg development remains controversial. In supporting the critical role of mTECs in Treg development, mice with disrupted thymic medulla, including *aly/aly* and *Traf6*$^{-/-}$ mice, have reduced numbers of $CD25^+CD4^+$ thymocytes and lower relative levels of Foxp3 mRNA [5,42]. Our unpublished data also demonstrate reduced Treg in the thymi of *Relb*$^{-/-}$ mice which lack medulla epithelium. However, cognate interactions with cortical epithelium were also reported sufficient for the phenotypic and functional development of $CD4^+CD25^+$ Treg [43,44]. A two-step Treg development model was recently proposed which reconciles to some degree the controversy discussed above. In this model, early TCR signaling results in the expression of proximal IL-2 signaling components which facilitate cytokine-mediated induction of Foxp3 [45–47]. Thus, it is likely that both cortex and medulla are sufficient to deliver TCR signaling while only medulla provides appropriate cytokines to com-

plete Treg development.

Among the medullary APCs, both mTECs and DCs seem sufficient for Treg development, since deletion of MHC-II on either mTECs or DCs has little effect on the number of thymic Tregs [44,48]. However, it is still unclear on the aspect of TCR specificity, whether mTECs matter given the fact they express numerous ectopic TRAs that stimulate TCR signaling. In fact, Aire promoter driven neo-self antigen expression in mTECs is sufficient to induce antigen specific Treg development [49]. It would be interesting to study whether lack of specific TRA in thymus would diminish antigen specific Treg development and confer to autoimmune response in the periphery.

4 TRA-independent roles of mTECs in central tolerance

In addition to ectopic TRA expression and antigen presentation, mTECs function in other ways. One important function is the expression of various chemokines. During thymocytes development, $CD4^+$ and $CD8^+$ single positive thymocytes have to migrate from cortex to medulla to undergo negative selection or further maturation before their emigration to periphery. Chemokine receptor CCR7 is important for SP thymocytes cortex-to-medulla migration, especially for $CD8^+$ SP thymocytes [50–53]. CCR7 ligands, secondary lymphoid tissue chemokine (SLC) and EBV-induced molecule 1 ligand chemokine (ELC), are mainly expressed in mTECs [54]. Disrupted CCR7-CCR7L axis results in partially defective negative selection and autoimmune response at periphery [51–53].

Not only cortex-to-medulla thymocyte migration is important for T cell development, intramedullary thymocyte scanning was recently suggested critical for efficient negative selection [39]. However, little is known about the regulation of intramedullary thymocyte migration. Does CCR7-CCR7L axis play a similar role there, since CCR7L seems regulated by Aire in mTECs [55]? Does the stable contact between thymocytes and APCs in medulla requires assistance from adhesion molecules, and if so, how are they regulated? These are interesting questions remaining to be investigated in the future.

5 The regulation of mTEC development

Given the critical role of mTECs in T cell development and central tolerance, it has been an attractive topic how this special type of cells develops and is well regulated. mTECs are believed to derive from common TEC progenitors, which can give rise to both cortical and medullary TEC lineages [56]. Although the existence of such bipotent progenitor cells is conclusive as demonstrated in elegant single cell tracing technique and clonal analysis [57,58], it remains a challenge to identify such TEC progenitors. This also impedes the study of regulation of differentiation from bipotent TEC progenitors to either mTECs or cTECs.

Even so, the past decade has seen tremendous progress in the mechanisms of mTEC development. Many molecules including Foxn1 and FGF have been found critical for both cTEC and mTEC development. This has been reviewed elsewhere [59]. In this article, we will focus on recent understanding of mTEC specific regulation, TNF receptor superfamily (TNFRSF) and NFκB pathway.

Serial studies of gene-deficient and mutant mice have revealed fragmentary mechanisms that regulate the formation and organization of mTECs, most of which are related to alternative NFκB pathways. *Ikka*$^{-/-}$, *Traf6*$^{-/-}$, *aly*/*aly*, *Relb*$^{-/-}$, and *Nfkb2*$^{-/-}$ mice display mTEC abnormalities and organ-specific autoimmunity [4–7,60,61]. *Ikka*$^{-/-}$, *aly*/*aly*, and *Relb*$^{-/-}$ strains of mice almost completely lack UEA-1^+ mTECs, suggesting a requirement for the alternative NF-kB pathways in mTECs. *Nfkb2*$^{-/-}$ mice, although with distinct cortical and medulla structures, possess autoimmune phenotypes, due to marked reduction of Aire and TRAs expression [61]. On the other hand, TRAF6 (a signal transducer activating the classical NFκB pathway) deficiency also leads to severe defects in thymus medulla as the alternative NFκB pathway-deficient strains do [5,62].

At the cellular receptor level, several molecules of TNFRSF may act as signal transducers activating the NFκB pathway. Deficiency of lymphotoxin beta receptor (LTβR), one upstream receptor of both classical and nonclassical NFκB pathways, results in a severe reduction of UEA-1^+ mTECs and reduced production of both Aire-dependent and Aire-independent TRAs [6,32,63]. Deficiency of CD40, another molecule of TNFRSF, leads to numerical and proportional reduction in $mTEC^{lo}$ subsets, but maintains normal medullary organization [60]. RANK signaling is strictly required for the UEA1^+ mTEC development in embryonic thymus [60]. Aire$^+$ and EpCAM$^+$ mTEC are also abolished in RANK deficient embryonic thymus [60]. However, RANK signaling seems to only partially contribute to the development or maintenance of mTECs in postnatal thymus.

Interestingly, LTβR, RANK and CD40 appear cooperately regulate mTEC development. Double deficiency of both RANK and CD40 resulted in more dramatic reduction of mTECs in postnatal thymus compared with single deficiency [60]. LTβR was also recently shown to synergize with RANK for mTECs developmental control by eliciting RANK expression in embryonic thymic stroma [64].

Who dictate mTEC development through those TNFRSF molecules mentioned above at the cellular level? Early study found that $CD4^+CD3^-$ lymphoid tissue inducer (LTi) cells could present RANKL for Aire-expressing mTEC development at the embryonic stage in an *in vitro* fetal thymic organ culture experimental model [20]. However, with the lack of obvious mTEC defects in Id2-deficient mice, which

lack LTi cells in adulthood, Takahama group argued LTi cells are dispensable for mTEC development in adult mice [65]. Instead, they identified positively selected thymocytes as the main source of RANKL for mTEC development in adult mice. More specifically, it seems that autoreactive $CD4^+$ thymocytes are important to control mature medullary thymic epithelial cell cellularity through antigen-specific interactions with mTECs (especially $mTEC^{hi}$ and $Aire^+$ $mTEC^{hi}$) [66]. Given the previous findings that $Aire^+$ mTECs have arrested proliferation and that Aire actually induces apoptosis in Aire-expressing mTECs [22], exactly how can self-reactive $CD4^+$ thymocytes promote mTEC development remains elusive.

Although much has been known about the molecular and cellular regulation of mTECs by TNFRSF and NFκB molecules, the exact mechanisms are still unclear, partially due to the lack of identity of bipotent TEC progenitors or mTEC precursor cells and the heterogeneity of mTECs. Further work is needed to dissect at which stage or in which subsets, namely, bipotent TEC progenitors, mTEC precursors, immature mTECs, Aire-null mature mTECs and $Aire^+$ mature mTECs, those molecules conduct their functions and how they control.

6 The role of mTEC during thymic involution and rejuvenation

Though a vital organ for homeostatic maintenance of the peripheral immune system, the thymus undergoes strikingly profound age-associated involution or atrophy, resulting in less efficient T-cell development, or thymopoiesis, and decreased emigration of naïve T cells. In addition, thymic atrophy has been noted in a number of physiological and pathological stress states including puberty and pregnancy, cancer, infection, chemotherapy, and ionizing irradiation exposure. Different from chronic age-associated atrophy, many of the stress induced thymic atrophies are transient and reversible. Under most circumstances, thymic decline is of minimal consequence to a healthy individual, however, may accumulatively contribute to the development of many diseases such as opportunistic infections, autoimmunity, inflammation and cancer. Furthermore, inability of adults to restore immune function after stresses may lead to increased morbidity and often mortality in the aged [67–69].

As to the underlying mechanisms of thymic involution, earlier studies attribute the age-associated involution to the loss of T cell progenitors or impaired TCR rearrangement [69]. However, in later studies, the role of thymic stroma in thymic involution gets more and more appreciated [70,71]. Many cytokines provided by thymic epithelial cells are found crucial for thymopoiesis; such cytokines include G-CSF, GM-CSF, interleukin 1 (IL-1), IL-3, IL-6, IL-7, macrophage-colony stimulating factor (M-CSF), stem cell factor (SCF), transforming growth factor β (TGF-β), oncostatin M (OSM), and leukemia inhibitory factor (LIF) [68,69]. The role of thymic stroma on thymic involution is further confirmed by genetic deficiency animal models. Age-related thymic involution is mediated by the sensitivity of sex hormones receptors in thymic epithelial cells [70] and can be reversed by physical or chemical blockade of sex hormones[71]. Recent study also reveals that thymic epithelial miR-29a is critical for diminishing the sensitivity of the thymic epithelium to simulated infection signals, protecting the thymus against inappropriate involution [72]. In addition, lymphoid tissue inducer cells derived IL-22 was found important for thymic regeneration after total body radiation induced thymic injury [73]. Administration of IL-22 enhanced thymic recovery after total body irradiation.

The importance of mTECs to thymic involution was recently recognized. In a model of endotoxemia-induced acute thymic involution and recovery, supraphysiologic leptin was demonstrated to play a role in protection of thymic epithelial cells [74]. The role of leptin is through its receptor which is restrictedly expressed in mTECs and IL-7, a critical cytokine for thymopoiesis produced by mTECs. In another physiological model, RhoB expression on mTECs was found important for thymus maintenance. Deficiency of RhoB led to earlier thymic atrophy, beginning as early as five weeks of age [75]. The enhanced expression of TGF-β receptor type II (TGFβRII) in thymic medullary epithelium was observed in RhoB deficient mice. Thus, RhoB may regulate thymus development and maintenance through the inhibition of TGF-β signaling in thymic medullary epithelium.

mTECs not only regulate thymic involution, their change during thymic involution may also have biological consequence given their important role for T cell development and function, an interesting argument that remains to be tested. If so, inhibiting mTECs involution or boosting its rejuvenation after stress maybe helpful for the establishment of central tolerance thus preventing autoimmune diseases in the aged, for the immune functional reconstitution during AIDS treatment to prevent deadly complications [76].

This work was supported by National Basic Research Program of China, Ministry of Science and Technology (2011CB946103) and National Natural Science Foundation of China (81261130022).

1 Fink P J, Bevan M J. H-2 antigens of the thymus determine lymphocyte specificity. J Exp Med, 1978, 148: 766–775
2 Kappler J W, Roehm N, Marrack P. T cell tolerance by clonal elimination in the thymus. Cell, 1987, 49: 273–280
3 Salaun J, Bandeira A, Khazaal I, et al. Thymic epithelium tolerizes for histocompatibility antigens. Science, 1990, 247: 1471–1474
4 Lomada D, Liu B, Coghlan L, et al. Thymus medulla formation and central tolerance are restored in IKKα/Mice that express an IKKα transgene in keratin 5^+ thymic epithelial cells. J Immunol, 2007, 178: 829–837
5 Akiyama T, Maeda S, Yamane S, et al. Dependence of self-tolerance on TRAF6-directed development of thymic stroma. Science, 2005, 308: 248–251

6 Boehm T, Scheu S, Pfeffer K, et al. Thymic medullary epithelial cell differentiation, thymocyte emigration, and the control of autoimmunity require lympho—epithelial cross talk via LTβR. J Exp Med, 2003, 198: 757–769
7 Zhu M, Fu Y X. Coordinating development of medullary thymic epithelial cells. Immunity, 2008, 29: 386–388
8 Viret C, Barlow A K, Janeway Jr C A. On the intrathymic intercellular transfer of self-determinants. Immunol Today, 1999, 20: 8–10
9 Klein L, Kyewski B. Self-antigen presentation by thymic stromal cells: a subtle division of labor. Curr Opin Immunol, 2000, 12: 179–186
10 van Meerwijk J P M, Marguerat S, Lees R K, et al. Quantitative impact of thymic clonal deletion on the T cell repertoire. J Exp Med, 1997, 185: 377–384
11 Klein L, Klein T, Rüther U, et al. CD4 T cell tolerance to human C-reactive protein, an inducible serum protein, is mediated by medullary thymic epithelium. J Exp Med, 1998, 188: 5–16
12 Klein L, Roettinger B, Kyewski B. Sampling of complementing self-antigen pools by thymic stromal cells maximizes the scope of central T cell tolerance. Eur J Immunol, 2001, 31: 2476–2486
13 Derbinski J, Schulte A, Kyewski B, et al. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. Nat Immunol, 2001, 2: 1032–1039
14 Aaltonen J, Bjorses P, Perheentupa J, et al. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. Nat Genet, 1997, 17: 399–403
15 Nagamine K, Peterson P, Scott H S, et al. Positional cloning of the APECED gene. Nat Genet, 1997, 17: 393–398
16 Anderson M S, Venanzi E S, Klein L, et al. Projection of an immunological self shadow within the thymus by the aire protein. Science, 2002, 298: 1395–1401
17 Liston A, Lesage S, Wilson J, et al. Aire regulates negative selection of organ-specific T cells. Nat Immunol, 2003, 4: 350–354
18 Gillard G O, Farr A G. Contrasting models of promiscuous gene expression by thymic epithelium. J Exp Med, 2005, 202: 15–19
19 Hamazaki Y, Fujita H, Kobayashi T, et al. Medullary thymic epithelial cells expressing Aire represent a unique lineage derived from cells expressing claudin. Nat Immunol, 2007, 8: 304–311
20 Rossi S W, Kim M Y, Leibbrandt A, et al. RANK signals from $CD4^+3^-$ inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. J Exp Med, 2007, 204: 1267–1272
21 Gäbler J, Arnold J, Kyewski B. Promiscuous gene expression and the developmental dynamics of medullary thymic epithelial cells. Eur J Immunol, 2007, 37: 3363–3372
22 Gray D, Abramson J, Benoist C, et al. Proliferative arrest and rapid turnover of thymic epithelial cells expressing Aire. J Exp Med, 2007, 204: 2521–2528
23 Koh A S, Kuo A J, Park S Y, et al. Aire employs a histone-binding module to mediate immunological tolerance, linking chromatin regulation with organ-specific autoimmunity. Proc Natl Acad Sci USA, 2008, 105: 15878–15883
24 Org T, Chignola F, Hetenyi C, et al. The autoimmune regulator PHD finger binds to non-methylated histone H3K4 to activate gene expression. EMBO Rep, 2008, 9: 370–376
25 Derbinski J, Kyewski B. How thymic antigen presenting cells sample the body's self-antigens. Curr Opin Immunol, 2010, 22: 592–600
26 Abramson J, Giraud M, Benoist C, et al. Aire's partners in the molecular control of immunological tolerance. Cell, 2010, 140: 123–135
27 van Attikum H, Gasser S M. Crosstalk between histone modifications during the DNA damage response. Trends Cell Biol, 2009, 19: 207–217
28 Ferguson B J, Alexander C, Rossi S W, et al. AIRE's CARD revealed, a new structure for central tolerance provokes transcriptional plasticity. J Biol Chem, 2008, 283: 1723–1731
29 Ramsey C, Bukrinsky A, Peltonen L. Systematic mutagenesis of the functional domains of AIRE reveals their role in intracellular targeting. Hum Mol Genet, 2002, 11: 3299–3308
30 Oven I, Brdičková N, Kohoutek J, et al. AIRE recruits P-TEFb for transcriptional elongation of target genes in medullary thymic epithelial cells. Mol Cell Biol, 2007, 27: 8815–8823
31 Giraud M, Yoshida H, Abramson J, et al. Aire unleashes stalled RNA polymerase to induce ectopic gene expression in thymic epithelial cells. Proc Natl Acad Sci USA, 2012, 109: 535–540
32 Chin R K, Zhu M, Christiansen P A, et al. Lymphotoxin pathway-directed, autoimmune regulator-independent central tolerance to arthritogenic collagen. J Immunol, 2006, 177: 290–297
33 Seach N, Ueno T, Fletcher A L, et al. The lymphotoxin pathway regulates aire-independent expression of ectopic genes and chemokines in thymic stromal cells. J Immunol, 2008, 180: 5384–5392
34 Klein L, Hinterberger M, von Rohrscheidt J, et al. Autonomous versus dendritic cell-dependent contributions of medullary thymic epithelial cells to central tolerance. Trends Immunol, 2011, 32: 188–193
35 Gallegos A M, Bevan M J. Central tolerance to tissue-specific antigens mediated by direct and indirect antigen presentation. J Exp Med, 2004, 200: 1039–1049
36 Koble C, Kyewski B. The thymic medulla: a unique microenvironment for intercellular self-antigen transfer. J Exp Med, 2009, 206: 1505–1513
37 Hubert F X, Kinkel S A, Davey G M, et al. Aire regulates the transfer of antigen from mTECs to dendritic cells for induction of thymic tolerance. Blood, 2011, 118: 2462–2472
38 Muller K P, Schumacher J, Kyewski B A. Half-life of antigen/major histocompatibility complex class II complexes *in vivo*: intra- and interorgan variations. Eur J Immunol, 1993, 23: 3203–3207
39 Le Borgne M, Ladi E, Dzhagalov I, et al. The impact of negative selection on thymocyte migration in the medulla. Nat Immunol, 2009, 10: 823–830
40 Fontenot J D, Rasmussen J P, Williams L M, et al. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. Immunity, 2005, 22: 329–341
41 Fontenot J D, Dooley J L, Farr A G, et al. Developmental regulation of Foxp3 expression during ontogeny. J Exp Med, 2005, 202: 901–906
42 Kajiura F, Sun S, Nomura T, et al. NF-kappa B-inducing kinase establishes self-tolerance in a thymic stroma-dependent manner. J Immunol, 2004, 172: 2067–2075
43 Bensinger S J, Bandeira A, Jordan M S, et al. Major histocompatibility complex class II—positive cortical epithelium mediates the selection of $CD4^+25^+$ immunoregulatory T cells. J Exp Med, 2001, 194: 427–438
44 Liston A, Nutsch K M, Farr A G, et al. Differentiation of regulatory $Foxp3^+$ T cells in the thymic cortex. Proc Natl Acad Sci USA, 2008, 105: 11903–11908
45 Burchill M A, Yang J, Vang K B, et al. Linked T cell receptor and cytokine signaling govern the development of the regulatory T cell repertoire. Immunity, 2008, 28: 112–121
46 Lio C W, Hsieh C S. A two-step process for thymic regulatory T cell development. Immunity, 2008, 28: 100–111
47 Hsieh C S, Lee H M, Lio C W. Selection of regulatory T cells in the thymus. Nat Rev Immunol, 2012, 12: 157–167
48 Hinterberger M, Aichinger M, da Costa O P, et al. Autonomous role of medullary thymic epithelial cells in central $CD4^+$ T cell tolerance. Nat Immunol, 2010, 11: 512–519
49 Aschenbrenner K, D'Cruz L M, Vollmann E H, et al. Selection of $Foxp3^+$ regulatory T cells specific for self antigen expressed and presented by $Aire^+$ medullary thymic epithelial cells. Nat Immunol, 2007, 8: 351–358
50 Ueno T, Saito F, Gray D H D, et al. CCR7 signals are essential for cortex-medulla migration of developing thymocytes. J Exp Med, 2004, 200: 493–505
51 Kurobe H, Liu C, Ueno T, et al. CCR7-dependent cortex-to-medulla migration of positively selected thymocytes is essential for establishing central tolerance. Immunity, 2006, 24: 165–177
52 Nitta T, Nitta S, Lei Y, et al. CCR7-mediated migration of

developing thymocytes to the medulla is essential for negative selection to tissue-restricted antigens. Proc Natl Acad Sci USA, 2009, 106: 17129–17133.
53 Zhu M, Chin R K, Tumanov A V, et al. Lymphotoxin β receptor is required for the migration and selection of autoreactive T cells in thymic medulla. J Immunol, 2007, 179: 8069–8075
54 Ueno T, Hara K, Willis M S, et al. Role for CCR7 ligands in the emigration of newly generated T lymphocytes from the neonatal thymus. Immunity, 2002, 16: 205–218
55 Laan M, Kisand K, Kont V, et al. Autoimmune regulator deficiency results in decreased expression of CCR4 and CCR7 ligands and in delayed migration of $CD4^+$ thymocytes. J Immunol, 2009, 183: 7682–7691
56 Anderson G, Lane P J L, Jenkinson E J. Generating intrathymic microenvironments to establish T-cell tolerance. Nat Rev Immunol, 2007, 7: 954–963
57 Bleul C C, Corbeaux T, Reuter A, et al. Formation of a functional thymus initiated by a postnatal epithelial progenitor cell. Nature, 2006, 441: 992–996
58 Rossi S W, Jenkinson W E, Anderson G, et al. Clonal analysis reveals a common progenitor for thymic cortical and medullary epithelium. Nature, 2006, 441: 988–991
59 Rodewald H R. Thymus organogenesis. Ann Rev Immunol, 2008, 26: 355–388
60 Akiyama T, Shimo Y, Yanai H, et al. The tumor necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. Immunity, 2008, 29: 423–437
61 Zhu M, Chin R K, Christiansen P A, et al. NF-κB2 is required for the establishment of central tolerance through an Aire-dependent pathway. J Clin Invest, 2006, 116: 2964–2971
62 Wang Y, Zhang P, Liu Y, et al. TRAF-mediated regulation of immune and inflammatory responses. Sci China Life Sci, 2010, 53: 159–168
63 Chin R K, Lo J C, Kim O, et al. Lymphotoxin pathway directs thymic Aire expression. Nat Immunol, 2003, 4: 1121–1127
64 Mouri Y, Yano M, Shinzawa M, et al. Lymphotoxin signal promotes thymic organogenesis by eliciting RANK expression in the embryonic thymic stroma. J Immunol, 2011, 186: 5047–5057
65 Irla M, Hugues S, Gill J, et al. Autoantigen-specific interactions with $CD4^+$ thymocytes control mature medullary thymic epithelial cell cellularity. Immunity, 2008, 29: 451–463
66 Irla M, Hugues S, Gill J, et al. Autoantigen-Specific Interactions with $CD4^+$ thymocytes control mature medullary thymic epithelial cell cellularity. Immunity, 2008, 29: 451–463
67 Tang H, Fu Y. A new role for T cells in dampening innate inflammatory responses. Sci China Life Sci, 2010, 53: 190–194
68 Lynch H E, Goldberg G L, Chidgey A, et al. Thymic involution and immune reconstitution. Trends Immunol, 2009, 30: 366–373
69 Taub D D, Longo D L. Insights into thymic aging and regeneration. Immunol Rev, 2005, 205: 72–93
70 Olsen N J, Olson G, Viselli S M, et al. Androgen receptors in thymic epithelium modulate thymus size and thymocyte development. Endocrinology, 2001, 142: 1278–1283
71 Sutherland J S, Goldberg G L, Hammett M V, et al. Activation of thymic regeneration in mice and humans following androgen blockade. J Immunol, 2005, 175: 2741–2753
72 Papadopoulou A S, Dooley J, Linterman M A, et al. The thymic epithelial microRNA network elevates the threshold for infection-associated thymic involution via miR-29a mediated suppression of the IFN-[alpha] receptor. Nat Immunol, 2012, 13: 181–187
73 Dudakov J A, Hanash A M, Jenq R R, et al. Interleukin-22 drives endogenous thymic regeneration in mice. Science, 2012, 336: 91–95
74 Gruver A L, Ventevogel M S, Sempowski G D. Leptin receptor is expressed in thymus medulla and leptin protects against thymic remodeling during endotoxemia-induced thymus involution. J Endocrinol, 2009, 203: 75–85
75 Bravo-Nuevo A, O'Donnell R, Rosendahl A, et al. RhoB deficiency in thymic medullary epithelium leads to early thymic atrophy. Int Immunol, 2011, 23: 593–600
76 Zhang L, Meissner E, Chen J, et al. Current humanized mouse models for studying human immunology and HIV-1 immuno-pathogenesis. Sci China Life Sci, 2010, 53: 195–203