Regulations of gene expression in medullary thymic epithelial cells required for preventing the onset of autoimmune diseases

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INTRODUCTION

The thymus contributes to self-tolerance of T cells by eliminating potentially self-reactive T cells and generating immunosuppressive T cells, which are essential for preventing the onset of autoimmune disease. Epithelial cells localized in the thymic medulla [medullary thymic epithelial cells (mTECs)] are non-hematopoietic in origin and play non-redundant roles in the elimination of self-reactive T cells (1–3). Recent studies have revealed that mTECs also contribute to the selection and survival of immunosuppressive Foxp3-positive regulatory T cells (Tregs) (4–8).

Medullary thymic epithelial cells express several functional molecules required for the selection of self-tolerant T cells and Tregs (9). Mature types of mTECs express MHC molecules and co-stimulatory molecules essential for antigen presentation to developing T cells. In addition, mTECs secrete several types of chemokines (e.g., CCL19, CCL21, and CCL22) that attract T cells or dendritic cells in the medulla (2, 9). Moreover, a recent study has shown that the expression of CD70 in mTECs enhances the development and survival of Tregs via an interaction with its receptor, CD27, which is expressed on thymic T cells (5).

A key feature of mTECs is their ability to express hundreds of self-antigens that are normally expressed in a tissue-specific manner (TSAs) (6, 10). TSAs are processed and directly presented by mTECs or indirectly presented by thymic DCs receiving TSAs from mTECs (4, 7, 11–13). T cells that recognize TSAs with high avidity undergo apoptosis (so-called negative selection) or survive as regulatory T cells (4, 14). Many studies have suggested significant roles of mTEC-dependent self-tolerance in preventing the onset of some autoimmune diseases in humans. Expression of some TSAs requires a nuclear protein autoimmune regulator (AIRE), the dysfunctional mutations of which are responsible for an inherited human autoimmune disease, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) (15, 16). Whereas the expression of AIRE mRNA is detected in different cell types, AIRE expression at the protein level is remarkably high in mTECs (17). A previous study using AIRE-deficient mice provided evidence that autoimmune, provoked by dysfunction of AIRE, is thymic stroma-dependent (18). In addition to APECED, recent studies have demonstrated that single-nucleotide polymorphisms (SNPs) in the AIRE gene are associated with rheumatoid arthritis (19, 20). In addition to mutations in the AIRE gene, reduced expression of the muscle acetyl choline receptor (CHRNA1) was shown to be associated with the onset of myasthenia gravis (21). Moreover, impairment of the mTEC-dependent tolerance might explain the relationship between myocarditis and autoimmunity (22). These findings also imply that the onsets of various human autoimmune diseases could be related to dysregulation of mTEC-dependent tolerance. Interestingly, in addition to relationships with autoimmune diseases, recent studies have uncovered roles for mTEC-dependent T-cell tolerance in tumor tolerance (8, 23, 24).

Because expression of AIRE and TSAs is characteristic of mTEC, mTECs should harbor specific mechanisms to direct AIRE and TSA expression. Expression of TSAs appears to be correlated...
with the differentiation of mTECs. In this mini-review, we spe-
cially focus on molecular mechanisms regulating the expression
of AIRE and TSAs and the process of mTEC differen-
tiation.

**DEVELOPMENT OF mTECs**

Thymic epithelial cells are classified into mTECs and cortical
thymic epithelial cells (cTECs) (2). Several lines of evidence
indicate the existence of a bi-potent TEC progenitor capable
of differentiating into mTECs and cTECs in the fetal and adult
thymus (25–29). The bi-potent TEC progenitor seems to give rise
to each progenitor of mTECs and cTECs in the next stage (30,
31). Recent studies revealed that mTECs differentiate from prog-
enitors expressing cTEC-markers (32, 33). These data imply that
mechanisms determining the mTEC commitment suppress the
cTEC-driving program. However, master molecules that decide
the fate of the bi-potent TEC progenitor expressing cTEC-markers
to the mTEC lineage have not been determined yet.

Currently, mTECs are classified based on the expression of
MHC II, CD80, AIRE, and involucrin (Figure 1). mTECs (typ-
ically defined as CD45− EpCAM+ Ly51− and UEA-1+ by flow
cytometric analysis) in adult mice are divided into two sub-
populations, according to the expression levels of MHC II and
CD80 (34). mTECs expressing high levels of MHC II and CD80
(mTEC hi) express a more diverse set of TSAs than mTECs expressing
lower levels of MHC II and CD80 (mTEC lo) do (35). Moreover,
precursor-product relationship analysis has suggested that the
mTEC hi fraction can differentiate into mTEC lo (36, 37). There-
fore, the mTEC hi fraction would be the more mature type of mTEC
than mTEC lo.

The mTEC hi fraction is further separated on the basis of AIRE
expression (36, 38). Because previous studies have indi-
cated that the AIRE-expressing mTEC hi (AIRE+ mTEC hi) are
postmitotic and susceptible to apoptosis (36), AIRE+ mTEC hi
are postulated to be the more differentiated cell types than
AIRE-negative mTEC hi. mTECs expressing involucrin, a marker
of terminally differentiated keratinocytes, are considered to be
terminally differentiated mTECs that may be derived from
AIRE+ mTEC hi (39, 40).

**REGULATION OF AIRE mRNA EXPRESSION**

Molecular mechanisms regulating the expression of AIRE, which
are likely critical for preventing autoimmunity, remain unclear.
In the fetal thymus, expression of AIRE starts at embryonic day
14.5 (41). Consistently, mature mTECs emerge around this embry-
onic day (42). Thus, AIRE expression seems to be closely linked
to mTEC differentiation. However, because mTEC hi is separated
into AIRE+ and AIRE− fractions, the mTEC differentiation me-
chanism might be necessary but is not entirely sufficient for AIRE
expression.

A study using a luciferase reporter assay identified a plausi-
ble minimal promoter region of the AIRE gene (43). This region
contains binding sequences for Sp1, AP-1, NF-Y, and ETS fam-
ily of transcription factors. Indeed, luciferase reporter analysis
suggested regulation of the AIRE gene promoter by ETS family
proteins (44). However, in vivo genetic studies are necessary to
prove that these sequence-specific transcription factors are critical
for the regulation of AIRE expression.

The promoter region of AIRE contains a high ratio of CpG sites
(43). These CpG sites are hypomethylated in established cell lines
defective in the AIRE expression. A subsequent study showed that
these CpG sites are hypomethylated in isolated mTECs compared
to thymocytes (45). These findings suggest that DNA demethyla-
tion might be prerequisite for AIRE expression. However, interest-
ingly, hypomethylation was also observed in cTECs and thymoma
with defective AIRE expression (45). Hence, DNA hypomethyla-
tion appears to be required but not sufficient for inducing AIRE
expression.

Overall, AIRE expression seems to be regulated by combi-
nations of chromatin modification and sequence-specific tran-
scription factors. However, precise mechanisms and regulatory
molecules remain to be determined.

**REGULATION OF TSA mRNA EXPRESSION**

TSA expression appears to be regulated by complicated mecha-
nisms. Single-cell PCR analyses revealed a stochastic nature of TSA
expression in mTECs (38, 46). Each TSA is expressed in a subset of
mTECs (38, 46). The frequency of mTECs expressing a particular
TSA was different, depending on the TSA (38, 46). Interestingly,
various combinations of TSAs are expressed in individual mTECs
(38, 46). These studies suggest that regulatory mechanisms of TSA
expression in mTECs are different from those used in inherent
tissues.

Several studies suggest that TSA expressions are epigenetically
controlled. A comprehensive mRNA expression study revealed
that TSA gene loci tend to co-localize in chromosomal clusters
(35, 47). Moreover, genomic imprinting of the Igf2 gene, a
TSA, was lost in mTECs (35), implicating the involvement of
a DNA demethylation mechanism in TSA expression. Interestingly, another imprinted gene, Cdkn1c, was not affected. These data imply the existence of mTEC-specific mechanisms for demethylation of DNA.

Control of TSA gene expression by AIRE has been extensively studied (48–50). Several studies have revealed a function of AIRE as a transcription factor that directly promotes TSA expression (51, 52). Furthermore, AIRE binds to hypomethylated Histone 3 Lys 4 (H3K4) through its plant homology domain (53, 54). This finding suggests that AIRE modifies the chromatin structure in the TSA genes. AIRE also binds to DNA-PK (55–57), which functions in the repair of DNA-double strand breakage. A study using an mTEC cell line suggested that interactions of AIRE with H3K4 and DNA-PK are critical in recruiting AIRE to TSA gene loci and promoting TSA expression (57). Additionally, it was reported that AIRE interacts with P-TEFb, a component of the super elongation complex (58). It is generally accepted that transcription elongation, via the release of “paused” RNA polymerase II, is critical for the regulation of many genes (58, 59). AIRE may recruit P-TEFb to the TSA gene locus and promote elongation of the arrested TSA transcripts by releasing RNA polymerase II from the proximal promoter (60). Recent comprehensive analysis of mRNA transcripts in mTECs supports this mechanism (61). In addition to the TSA expression, the AIRE-dependent expression of some microRNAs (miRNAs) was recently revealed (62, 63). Consistently, genetic studies revealed important roles played by miRNA expressions in functions and maintenance of mTECs (63–65).

Compared to the mechanisms underlying Aire-dependent TSA expression, molecular mechanisms underlying Aire-independent TSA expression are less understood. As described above, whereas epigenetic regulations of TSA genes would be critical, mechanisms underlying epigenetic changes specific for mature mTECs remain unclear. Moreover, unidentified transcription factors may be involved in the promotion of Aire-independent TSA expressions.

**EXTRACELLULAR SIGNALING TO PROMOTE DIFFERENTIATION OF mTECs EXPRESSING AIRE AND TSAs**

Differentiation of TECs is well known to be correlated to differentiation of T cells in the thymus (so-called thymic cross-talk) (3). mTEC maturation was reported to be abolished in severe combined immunodeficiency (SCID) patients (66). This finding supports the idea that failure of the thymic cross-talk results in the onset of autoimmunity manifesting through inhibition of mTEC function. Interestingly, a recent study showed that administration of anti-CD3ε antibody ameliorated autoimmunity in leaky SCID model mice possibly through improvement of the thymic cross-talk (67).

Molecular basis of the thymic cross-talk in mTEC development has been reported. Several lines of evidence revealed that TNF family cytokines expressed in thymocytes and other cells of hematopoietic origin (2) and their receptors expressed in mTEC are critical for the thymic cross-talk. Briefly, signaling of TNF receptor family members, RANK, CD40, and lymphotixin-β receptor (LbR), play essential roles in the development of mTECs expressing Aire and TSAs. This topic has been summarized in a recent review (1).

**DOWNSTREAM OF TNF RECEPTOR FAMILY SIGNALING**

TNF receptor family signaling induces the activation of NF-κB and MAPK pathways (68). To date, the involvement of the MAPK pathway in the development of mTEC remains to be addressed. However, several lines of evidence have indicated that the NF-κB family plays a critical role in the development of mTECs expressing AIRE and TSAs.

NF-κB members are sequestered in the cytoplasm in an inactive state by the binding of the inhibitory protein IκB in resting cells (69–71). Lignations of receptors induce phosphorylation and subsequent degradation of IκB proteins, thereby leading to nuclear localization of NF-κB to activate transcription. Two distinct NF-κB activation pathways, the classical pathway and the non-classical pathway, are currently known (70–72) (Figure 2). The classical pathway is required in inflammatory responses and lymphocyte activation (71). On the other hand, the non-classical pathway mainly promotes development and architecture formation of lymphoid organs, including the thymus. In the non-classical pathway, receptor ligation induces accumulation of the NF-κB-inducing kinase (NIK), which is normally degraded by the ubiquitin-dependent proteasome in resting cells. Subsequently, accumulated NIK phosphorylates and activates IKKα, which induces partial degradation of p100 to p52. p100 preferentially binds to and sequesters RelB in the cytoplasm, and the partial degradation of p100 to p52 induces translocation of RelB and p52 as a heterodimer into the nucleus.

The requirement for NF-κB activation in the development of mTEC was initially identified by the analysis of RelB-deficient mice (73, 74). RelB-deficient mice showed severe reduction in medulla size, accompanied by a lack of UEA-1-positive mTECs. Consistently, the expression of AIRE was abolished in the RelB-deficient thymus (6, 41, 75). As expected, RelB-deficient mice showed severe autoimmune diseases. A recent study demonstrated that autoimmunity of RelB mice was due to the defect in thymic stroma function (6). Mice carrying a dysfunctional mutation, NIK (aly/aly), also showed a similar defect in mTEC development and autoimmune phenotypes (76–78). Whereas IKKδ-deficient mice die shortly after birth, neonatal IKKα-deficient mice and transplantation of IKKα-deficient thymic stroma indicates a requirement of IKKα in the development of mTECs (79, 80). mTEC development in p100-deficient mice is partially defective (81, 82), but this appears to be due to a partial rescue of p100 function by p105 (or its processed product, p50) because the double deficiencies of p100 and p105 resulted in severe defects in mTEC development, similar to the RelB- and NIK-mutant mice (83). Overall, these results support the idea that activation of the non-classical NF-κB pathway is essential for the development of mTECs.

**TRAF6** is a signal transducer that mediates signaling from TNF receptor family members (84, 85). TRAF6-deficient mice exhibit severe autoimmune disease (86, 87). Additionally, recent studies suggest possible associations between SNPs of the TRAF6 gene with rheumatoid arthritis and systemic lupus erythematosus in humans (88, 89). Previous studies showed that TRAF6 promotes the development of mTECs expressing AIRE and TSAs, thereby suppressing autoimmunity (86). Moreover, RANK-mediated differentiation of mTECs requires TRAF6 in in vitro organ culture.
Imply a role for TRAF6-mediated activation of the classical NF-κB pathway in mTEC differentiation. Notably, TRAF6 is a signal transducer and activator of transcription (STAT) family member, which is involved in the NF-κB signaling cascade. This pathway mediates the expression of cytokines and chemokines that promote T cell differentiation and survival. In the context of mTECs, TRAF6 activation is critical for the expression of AIRE and TSAs, which are important for central tolerance and the establishment of regulatory T cells (Tregs).

CONCLUDING REMARKS

Whereas significant roles for NF-κB in signal activation of mTECs have been established, recent findings suggest that other signaling pathways, such as the JAK/STAT and PI3K/AKT pathways, also play crucial roles in mTEC development and function. The interplay between these pathways is complex and requires further investigation to fully understand the molecular mechanisms underlying mTEC differentiation.

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