Expression of Self-antigen in the Thymus: A Little Goes a Long Way

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Tissue Antigens Are Not Sequestered but Are Expressed in Thymus. For many years, it has been hypothesized that a major factor in organ-specific autoimmunity is a lack of central tolerance to self-antigens that are sequestered behind anatomical barriers in organs such as the eye, testis, and brain. However, numerous studies published in the last few years argue strongly that this concept of sequestration of tissue-specific antigens in such immune-privileged organs should be revised. Many tissue-specific antigens are now known to be expressed in the thymus (for review see 1) primarily in medullary thymic epithelial cells (mTECs) (2), which appear to be a specialized subset of cells that may mediate deletion of self-reactive T cells and/or the induction of regulatory T cells (Tregs). Genetic analysis of a group of human autoimmune diseases known as autoimmune polyendocrinopathy-candidiasis-ectodermaldystrophy (APECED) has shown that the expression of tissue antigens in mTECs may be mediated by a transcription factor called AIRE (autoimmune regulator). Both mice and humans deficient in AIRE develop a polyglandular disorder characterized by autoimmunity involving the parathyroid and/or adrenal glands (3). AIRE, which is expressed predominantly in mTECs, has now been demonstrated to act as a master regulator of the expression of many tissue-specific antigens, including antigens that are targeted in APECED, such as salivary proteins 1 and 2 (4). How AIRE mediates transcription of all these tissue-specific antigens in mTECs has not been elucidated. It is important to note that although AIRE regulates thymic expression of ~100–300 genes, AIRE deficiency results in autoimmunity directed to a restricted set of antigens. It is therefore tantalizing to speculate whether paralogs of AIRE exist that control thymic expression of a distinct cohort of tissue-specific antigens that are involved in other autoimmune diseases.

Thymic Expression of Tissue Antigens Shapes the Self-reactive Repertoire. The fact that tissue-specific antigens are expressed in the thymus makes less tenable the hypothesis that autoimmunity results from a failure to impose central tolerance on the developing T cell. Recent studies in different animal models of autoimmunity have shed some light on how tolerance is achieved and subverted in individuals where the target antigen is expressed in the thymus. Here, we illustrate three different scenarios that arise from these studies.

In the first scenario, self-antigen expressed in the thymus deletes high affinity self-reactive T cells, whereas low affinity T cells are seeded to the periphery. Evidence for this comes from studies of the T cell repertoire specific for myelin basic protein (MBP), which is a target antigen in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. MBP-deficient, C3H (H-2^k) mice immunized with whole MBP, exhibit a strong T cell response to MBP 79–87, whereas WT mice exhibit a response that is at least 3 logs lower in avidity (5).

In the second scenario, self-reactive cells escape thymic deletion because the isoform of the antigen expressed in the thymus differs from that expressed in the target organ. This is best illustrated by studies of the T cell repertoire specific for myelin proteolipid protein (PLP), another target antigen in EAE. The predominant form of PLP expressed in the thymus is a splice variant, DM20, that lacks residues 116–150 of the full-length protein (6,7). Thus, only cells that react with epitopes within DM20 would be expected to undergo central tolerance. Indeed, this has been shown to be the case in studies of the T cell repertoire to PLP in WT versus PLP-deficient C57BL/6 mice (7).

In the last scenario, thymically expressed self-antigens do not mediate central tolerance due to inefficient antigen presentation by MHC alleles present in the individual. This mechanism has been proposed to explain the escape from tolerance of MBP 1–11–reactive T cells. MBP 1–11, which is expressed in the thymus (8), has been shown to bind weakly to I-A^u (9) and to form unstable peptide–MHC complexes (10), resulting in the escape of self-reactive cells from thymus. In addition, MHC instability and poor peptide binding may also underlie the susceptibility of nonobese diabetic (NOD) mice to autoimmunity. In the NOD strain, homozygosity for the I-A^{g7} allele is strongly associated with susceptibility to type-1 diabetes. This MHC molecule has been shown to be structurally unstable and to bind disease-related self-peptides poorly (11–13). Collectively, these examples demonstrate that the expression of self-antigen in thymus shapes the self-reactive repertoire, but in some cases deletion may not be complete or may not occur at all

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due to the limited affinity of the MHC molecule for the self-antigen or due to expression of a splice variant or isoform of the self-antigen.

How Much Antigen Must Be Expressed in the Thymus? All of the mechanisms illustrated above involve expression of self-antigen in the thymus. What about the cases where thymic expression of the self-antigen has not been detected by standard techniques? In this issue, Avichezer et al. examine the effects of loss of expression of interphotoreceptor binding protein (IRBP), a target antigen in experimental autoimmune uveitis (EAU), on the T cell repertoire (14). Previous examination using RT-PCR and immunoblotting techniques had shown that IRBP expression in the thymus correlates with disease susceptibility and resistance to EAU, with resistant strains of mice exhibiting detectable IRBP and susceptible strains lacking expression (15). Surprisingly, Avichezer et al. demonstrate that IRBP deficiency in the susceptible strain B10.RIII, which had been shown previously to lack detectable IRBP expression in the thymus, has dramatic effects on the T cell repertoire to IRBP. The authors find that the T cell response to the immunodominant epitope of IRBP is augmented and that additional T cell epitopes are uncovered in IRBP-deficient mice. This prompted the authors to use ultrasensitive immunohistochemistry coupled with RT-PCR analysis of microdissected tissue to demonstrate that IRBP is in fact expressed in the thymus of B10.RIII mice. Interestingly, IRBP expression is seen in very small clusters of cells located in the medulla and cortico–medullary junction (14). Although the authors do not formally identify the cell type that expresses IRBP, it is possible that they are mTECs.

These results suggest that a seemingly miniscule amount of self-antigen expression can have a dramatic effect on self-tolerance and that central tolerance should not be ruled out in cases where attempts to detect expression of self-antigen in the thymus by conventional techniques have yielded negative results. This raises the question of how do all of the millions of self-reactive T cells generated in the thymic cortex come in contact with the few mTECs expressing self-antigen? It is possible that mTECs produce factors that specifically attract developing thymocytes to them, thereby increasing the likelihood that cells bearing deleterious self-reactive receptors will come in contact with self-antigen and undergo tolerance.

That ectopic thymic expression of tissue-specific antigens is not absolutely required for shaping the self-reactive T cell repertoire is illustrated in another model of MBP-induced EAE. In MBP-deficient mice on the H-2^u background, the immunodominant epitope is MBP 121–150. T cells reactive to this epitope are virtually undetectable in WT mice, suggesting that they undergo central tolerance (10). Given that MBP 121–150 is not synthesized in the thymus (8, 16, 17), how could central tolerance be elicited? It has now been shown that BM-derived antigen-presenting cells acquire MBP 121–150 exogenously, carry it into the thymus, and mediate central tolerance (18). Therefore, lack of thymic expression of self-antigen may not always preclude deletion of self-reactive T cells. Although exogenous self-antigen that is carried to the thymus can mediate central tolerance by deletion in thymus, it is not known if it also has a role in the generation of Tregs or if the generation of Tregs is a unique property of self-antigen synthesized in the thymus by mTECs.

Thymus Is the Nursery for the Generation of Regulatory T Cells. Although for many years the major role of the thymus was considered to be the deletion of self-reactive cells, evidence existed in the literature that the thymus is also the seat of the generation of regulatory T cells (19–21). It had been shown previously that the thymus was very efficient in generating regulatory T cells (20) and that mice thymectomized 3 d after birth develop multiorgan autoimmune diseases (22). In this regard, Avichezer et al. show that depletion of $CD25^+$ Tregs increases susceptibility to EAU and that these regulatory cells are thymus derived (14). Although the authors clearly show that Tregs have a role in IRBP-induced EAU, they do not directly address whether they are dependent on IRBP for their generation or are specific for IRBP. The small amount of IRBP present in the thymus appears to be sufficient to eliminate some IRBP specificities, but does it have a role in generating Tregs? Assuming it does, would even lower expression of IRBP render deletion of the T cell repertoire to IRBP ineffective but still result in the generation of the Tregs? In other words, is there an amount of self-antigen expression that is sufficient for the generation of Tregs but not sufficient for the deletion of self-reactive T cell specificities or vice versa? Consistent with the idea that this is a quantitative issue is the suggestion that the affinity of Tregs teeters on the edge of the range of affinities that would be expected to undergo negative selection in the thymus (23). If this is the case, then why are these cells not deleted but sustained and exported to the periphery? The solution to this conundrum might lie in understanding the expression pattern and function of the transcription factor Foxp3. Foxp3 is up-regulated in Tregs and is involved in inhibiting many T cell functions including the production of cytokines (24–26). It is possible that self-reactive T cells that come in contact with their cognate antigen in thymus avoid deletion by up-regulation of Foxp3 and thus become Tregs. Other cells that are not able to up-regulate expression of Foxp3 are purged from the repertoire. Another possibility is that Tregs are a different lineage of T cells that develop and mature by coming in contact with self-antigens in the thymus; however, there is little support for this proposition.

What emerges is an interesting picture of how the expression of tissue-specific antigens in the thymus can impose tolerance by different means: deletion of self-reactive T cells and induction of regulatory T cells. Two different transcription factors, AIRE and Foxp3, have been shown to play a crucial role in imposing this tolerance, one (AIRE) regulates the expression of tissue antigens in the thymus and the other (Foxp3) plays a crucial role in the induction of anergy and generation of Tregs. Natural mutations of both of these transcription factors in humans and mice induce devastating multiorgan autoimmune diseases, but the phenotype resulting from Foxp3 deficiency is far more severe (3, 27). This raises the important question of

whether the principal function of the thymus is in generating Tregs and whether this function is more important than its role in purging self-reactive T cells from the developing repertoire. Additionally, the phenotype resulting from Foxp3 deficiency is also more severe than that resulting from depletion of $CD4^+CD25^+$ Tregs. This could suggest that Foxp3 also plays a role in other mechanisms of central tolerance or in generating regulatory T cells that are not defined by the $CD4^+CD25^+$ phenotype.

Although AIRE and Foxp3 are important clues in the quest to unravel the mechanisms of negative selection and generation of Tregs in the thymus, our knowledge of these processes is still in its infancy. The next breakthrough may lie in answering the most burning question of all: what is the antigen specificity of Tregs? Although induced by exposure to self-antigen in the thymus, we may find that Tregs are not specific but are broadly cross-reactive to multiple self-antigens and thus provide a protective shield by inhibiting immune responses to self and preventing immunopathology mediated by nonself.

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