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The Sickness Unto DEAF

Abstract

While promiscuous expression of tissue-specific antigens (TSAs) in the thymus is essential for self-tolerance, recent evidence suggests that immunologically relevant TSA expression may also occur in the secondary lymphoid organs. A new study links the transcriptional regulator DEAF-1 with altered TSA expression in the secondary lymphoid organs and autoimmune diabetes.

Immunologic self-tolerance requires the continuous vigilance of overlapping but non-redundant mechanisms to prevent the escape and activation of pathogenic autoreactive lymphocytes. Known mechanisms include, but are not limited to, the generation of regulatory T (T_{reg}) cells, the requirement for a second signal for activation of naive T cells, T cell subset skewing by the cytokine milieu, immunologic sequestration of certain organs, and negative selection of autoreactive thymocytes. That such a diverse array of mechanisms must all work in concert to prevent autoimmunity highlights the complexity of the problem of self versus non-self discrimination, and the delicate balance that exists between robust pathogen elimination and self-reactivity. It is widely accepted that promiscuous tissue specific antigen (TSA) gene expression in thymic stroma is essential for immune tolerance. Recent evidence suggests that self-antigen-expressing stroma in secondary lymphoid organs may serve as an additional checkpoint to eliminate or shut down self-antigen-specific T cells that escape negative selection in the thymus. The immunological relevance of this phenomenon remains unclear, but a study by Yip *et al.* in this issue of *Nature Immunology* provides further support for the notion that secondary lymphoid organs are sites of promiscuous TSA gene expression, and that fluctuations in TSA expression in the pancreatic lymph node (PLN) correlate with progression toward autoimmune type 1 diabetes (T1D) in non-obese diabetic (NOD) mice. In addition, Yip *et al.* identify the SAND domain-containing transcriptional regulator Deformed epidermal autoregulatory factor 1 (DEAF-1) as a potential mediator of this process.

When pancreas-specific promoters were first used to drive expression of T cell antigens in transgenic mice, a number of groups reported transgene expression in the thymus in addition to the expected expression in the target tissue; this thymic expression resulted in T cell tolerance to these antigens^{1, 2}. It soon became clear that thymic medullary epithelial cells express low amounts of antigens representing a wide range of tissues³. The subsequent discovery that much of this thymic promiscuous gene expression depended on the transcriptional regulator AIRE, and that mice and humans lacking functional AIRE develop devastating autoimmunity^{4, 5}, lent credence to the idea that a faithful thymic representation of the peripheral self is essential for normal immune homeostasis.

More recent evidence indicates that something similar may occur in the secondary lymphoid organs. Lee *et al.*⁶, using the intestinal fatty acid binding protein promoter to drive ovalbumin (OVA) expression, found that the lymphoid stroma, particularly a fibroblastic lymph node stromal cell (LNSC) population, promiscuously express OVA as well as a host of other tissue-specific transcripts which in turn leads to deletion of adoptively transferred OVA-specific CD8 T cells. Similarly, the endogenous melanocyte antigen tyrosinase is expressed by lymphoid stroma, and causes deletion of cognate CD8 T cells that are not negatively selected in the thymus⁷. More recent work described a population of extrathymic *Aire*-expressing cells (eTACs) in the peripheral lymphoid stroma that express a host of AIRE-regulated TSAs distinct from those regulated by AIRE in the thymus, and whose interaction with naive cognate CD8 T cells leads to deletion⁸.

The report by Yip *et al.* supports the idea that TSA expression occurs in the secondary lymphoid organs, and identifies a novel putative regulator of such TSA expression, DEAF-1. Based on their previous studies⁹, the authors used microarrays to follow changes in gene expression in whole PLN of NOD mice before, during, and after disease onset. Comparison of these transcriptional profiles to the congenic but non-diabetic NOD.B10 strain revealed that the PLN of NOD mice expressed a range of TSA transcripts including insulin (*Ins2*), pancreatic polypeptide (*Ppy*), and regenerating islet-derived 3 gamma (*Reg3g*); the expression of these transcripts in diabetic mice fluctuates coordinately in concert with disease progression and is generally low during the approximate window of disease onset. As DEAF-1 expression in the PLN fluctuates in parallel with TSA expression, and DEAF-1 bears a general structural similarity to AIRE, Yip *et al.* chose to further investigate the role of DEAF-1 in peripheral TSA expression.

DEAF-1 is a DNA-binding protein first identified in *Drosophila* as a regulator of the homeotic gene Deformed¹⁰, which facilitates anterior segment development. Loss-of-function mutations in *Drosophila* DEAF-1 lead to segmentation defects and death during embryonic development, whereas DEAF-1 overexpression leads to altered wing and eye formation¹¹. The murine ortholog *Deaf-1* is expressed in a wide variety of fetal and adult tissues¹². Mice lacking functional *Deaf-1* exhibit a range of anterior body plan defects including exencephaly, failure of neural tube closure, and skeletal abnormalities in the rib cage and cervical vertebrae¹³. *Deaf-1* is also widely expressed in the adult mouse, and regulates the proliferation and branching of mammary epithelium¹⁴.

To more directly test the role of DEAF-1 in TSA expression, Yip *et al.* analyzed by microarray whole PLN from wild-type and *Deaf-1*-deficient mice. This analysis indicated that DEAF-1 positively and negatively regulates the expression of a host of genes including a significant number of TSAs in the PLN. Strikingly, 22 of the 30 most highly DEAF-1-induced genes are tissue-specific, and the set of DEAF-1-regulated TSAs identified by microarray shares some overlap with AIRE-regulated genes in the thymus, though *Aire* transcription itself is unchanged in *Deaf-1*-deficient LN. Conversely, the set of DEAF-1-regulated genes in the PLN is almost entirely distinct from the set of genes regulated by AIRE in the secondary lymphoid organs⁸, suggesting that these systems may be independent or complementary. Similarly, knockdown of DEAF-1 *in vitro* caused reduced expression of candidate TSAs. Finally, although no organ-specific autoimmune infiltration or tissue destruction was observed in mice lacking DEAF-1, modest nonspecific antibody reactivity against retinal tissues was detected. The absence of overt autoimmunity in these mice may be partly due to the BALB/c genetic background, but suggests that whatever defects in self-tolerance exist in the absence of DEAF-1 may be subtle.

On closer examination, Yip *et al.* also detected a novel DEAF-1 splice variant (DF1-VAR1). This variant, enriched in the PLN of pre-diabetic NOD mice, contains an intronic insertion that introduces a premature stop codon leading to loss of the nuclear localization signal (NLS) as well as other C-terminal domains of the protein. Unlike canonical DEAF-1, the splice variant is largely sequestered in the cytoplasm *in vitro*, and can bind to canonical DEAF-1, potentially interfering with its ability to induce transcription. A similar NLS-deficient splice variant was identified in the PLN of humans and, intriguingly, this variant is significantly enriched in the PLN of T1D patients. The authors hypothesize that this splice variant may interfere with the normal function of DEAF-1, leading to reduced expression of DEAF-1-dependent TSA genes in the PLN at critical points during T1D progression.

Taken together, these results raise the interesting prospect that TSA expression in the PLN may facilitate protection from T1D, and that DEAF-1 may play an important role in this process. However, a number of important questions and considerations remain. The identity of the *Deaf-1*-expressing cells involved in this process, and their relationship to previously

characterized stromal populations including including UEA-1⁺ gp38⁺ LNSCs⁶ and Aire-expressing eTACs⁸, will be of significant interest (Fig. 1). The current study for the most part examines TSA expression in whole LN, although Yip *et al.* do document *Deaf-1* and TSA expression in CD45⁺ lymph node stromal elements (LNSE). However *Deaf-1*, unlike *Aire*, is expressed in a wide array of tissues, and the precise relationship between *Deaf-1*-expressing LNSE and previously described populations in the lymphoid organs remains to be defined. It will also be informative to determine whether DEAF-1 regulates TSA expression directly. Unlike AIRE, DEAF-1 has direct DNA-binding ability in its SAND domain, and discrete DEAF-1 response elements have been identified¹⁵. It will be interesting, therefore, to determine whether DEAF-1-regulated TSAs contain such response elements and whether interaction between DEAF-1 and TSA promoter regions can be detected by chromatin immunoprecipitation.

Further, it is important to note that despite the potential promise of secondary lymphoid TSA expression, the biological relevance of this phenomenon remains largely speculative. To our knowledge no study has yet shown directly that promiscuous TSA expression in the secondary lymphoid stroma is either necessary or sufficient to prevent autoimmunity. While the present study identifies a mild predisposition toward autoantibody reactivity among *Deaf-1*-deficient mice, it is not yet clear whether this defect maps to secondary lymphoid organs and a defect in TSA expression, or whether it reflects part of the larger developmental disruption seen in the setting of systemic *Deaf-1*-deficiency. Indeed, while the decrease in TSA and *Deaf-1* expression in the PLN of NOD mice at 12 weeks provides evidence for a correlation between promiscuous gene expression and autoimmunity, the causal relationship between the two is less clear. To this end, it will also be important to directly demonstrate that DEAF-1-dependent alterations in TSA expression have functional consequences for peripheral T cell selection and/or function. Ultimately we must determine whether and why such TSA expression in the secondary lymphoid organs is necessary given its clear role in the thymus and the efficiency of thymic negative selection. One answer may lie in the complementary rather than redundant nature of central and peripheral TSA expression.

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Figure 1.

Putative TSA-expressing stromal populations in the LN. *Deaf-1* expression is described in CD45⁻ LN stromal elements (LNSE). Previously defined TSA-expressing stromal cell populations include gp38⁺ UEA-I⁺ LN stromal cells (LNSCs) and EpCAM⁺, UEA-I⁻ gp38⁻ extrathymic *Aire*-expressing cells (eTACs). The relationship of these populations to one another and the respective roles of AIRE and DEAF-1 in regulating expression of TSAs in the secondary lymphoid organs remain unknown.