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Commentary

Genetic alterations are known drivers of autoimmune disease; however, there is a much higher incidence of autoimmunity in women, implicating sex-specific factors in disease development. The autoimmune regulator (*AIRE*) gene contributes to the maintenance of central tolerance, and complete loss of *AIRE* function results in the development of autoimmune polyendocrinopathy syndrome type 1. In this issue of the *JCI*, Dragin and colleagues demonstrate that *AIRE* expression is downregulated in females as the result of estrogen-mediated alterations at the *AIRE* promoter. The association between estrogen and reduction of *AIRE* may at least partially account for the elevated incidence of autoimmune disease in women and has potential implications for sex hormone therapy.

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Genetic alterations are known drivers of autoimmune disease; however, there is a much higher incidence of autoimmunity in women, implicating sex-specific factors in disease development. The autoimmune regulator (AIRE) gene contributes to the maintenance of central tolerance, and complete loss of AIRE function results in the development of autoimmune polyendocrinopathy syndrome type 1. In this issue of the *JCI*, Dragin and colleagues demonstrate that AIRE expression is downregulated in females as the result of estrogen-mediated alterations at the *AIRE* promoter. The association between estrogen and reduction of AIRE may at least partially account for the elevated incidence of autoimmune disease in women and has potential implications for sex hormone therapy.

Autoimmune disease: sex-dependent differences

The incidence of autoimmune disease is increasing worldwide; therefore, the need to understand the basis of autoimmunity has taken on a new urgency. Progress in identifying genetic contributors to autoimmunity has been made through the study of monogenic autoimmune diseases. Such an approach has identified critical immune-regulatory genes, such as the autoimmune regulator (*AIRE*), which encodes a nuclear protein that functions as a key regulator of thymic central tolerance (reviewed in ref. 1). *AIRE* enforces self tolerance by promoting the promiscuous expression of tissue self-antigens (TSAs) within medullary thymic epithelial cells (mTECs), a nonhematopoietic, stromal cell population (Figure 1A). Presentation of these TSAs within the thymus results in negative selection of bone marrow-derived T cells, which recognize these TSAs with high affinity. In addition to eliminating autoreactive T cell clones within the thymus, *AIRE* also maintains self tolerance by diverting autoreactive T cells into the Treg lineage. *AIRE* is important for preventing autoimmune disease, as individuals with a complete loss of *AIRE* function develop the multiorgan

autoimmune disease autoimmune polyendocrinopathy syndrome type 1 (APS1, which is also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy [APECED]).

While genetics are a major factor that determines autoimmune disease predisposition, it is clear that sex also plays a defining role in disease development. The incidence of autoimmunity is skewed toward females for many autoimmune diseases, including Sjögren's disease, systemic lupus erythematosus (SLE), and autoimmune thyroid disease (reviewed in ref. 2). Remarkably, over 80% of those affected with these conditions are female, illustrating the strong influence of sex in disease predisposition. Multiple factors likely underlie this sex bias, including the distinct sex hormone profiles in females versus males. Accumulating evidence suggests that male androgen hormones are immune suppressive, while female estrogen hormones are immune activating. For instance, androgen increases the differentiation of immunoregulatory CD4⁺CD25⁺FOXP3⁺ T cells (3), whereas estrogen enhances survival of T cells in patients with autoimmunity (4). Thus, there is a plethora of evidence that sex hormones directly modulate T cells in the periphery.

Early clues that sex hormones may also modulate thymic stromal populations, such as mTECs, came from elegant studies that utilized bone marrow chimeras (5, 6). These studies showed that thymic epithelial cells express estrogen receptor (ER) and androgen receptor (AR) and that expression of these hormone receptors in the stromal compartment is required for altering bone marrow-derived T cell subsets in the thymus (5, 6). In this issue, Dragin et al. confirm that sex hormones indeed act on receptors on thymic stromal cells to impinge upon T cell development within the thymus. Furthermore, this study demonstrates that sex hormones regulate *AIRE* expression in mTECs and, in particular, estrogen decreases *AIRE* to predispose females to autoimmunity (7).

Estrogen-mediated *AIRE* downregulation

Transcriptional control of *AIRE* is complex and involves cis-regulatory elements and epigenetic modifications (Figure 1A). Identified regulators include enhancer elements that contain NF-κB response elements (8, 9); transcription factors, including activator protein-1 (AP-1), specificity protein 1 (Sp1), nuclear factor Y (NF-Y), and ETS family transcription factors (10); histone modifications (9); and DNA methylation (11). Dragin et al. report that direct binding of estrogen/ER complexes to the *AIRE* promoter is unlikely, as predicted estrogen response elements are lacking at this site. Instead, estrogen-dependent downregulation of *AIRE* transcription was determined to be dependent on DNA methylation, an epigenetic mark of gene silencing (Figure 1B). An inhibitor of DNA methyltransferases (DNMTs), 5-aza-2'-deoxycytidine, blocked estrogen-mediated modulation of *AIRE*, and addition of estrogen to primary human mTEC cultures increased DNA methylation at the *AIRE* promoter. Notably, it remains to be clarified exactly how estrogen promotes DNA methylation. One could speculate that estrogen/ER complexes may promote DNMT function to increase DNA methylation.

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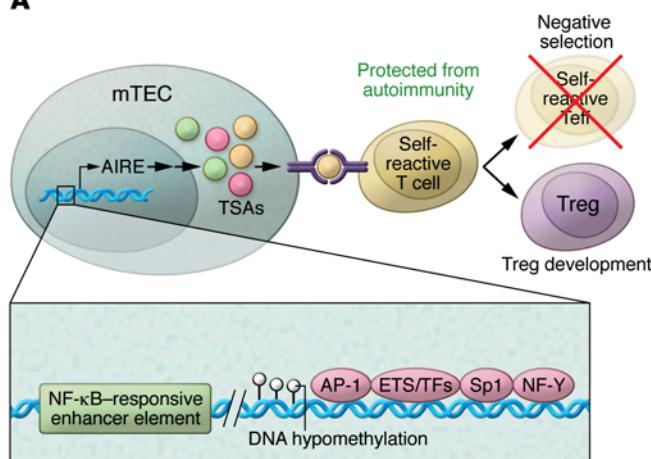
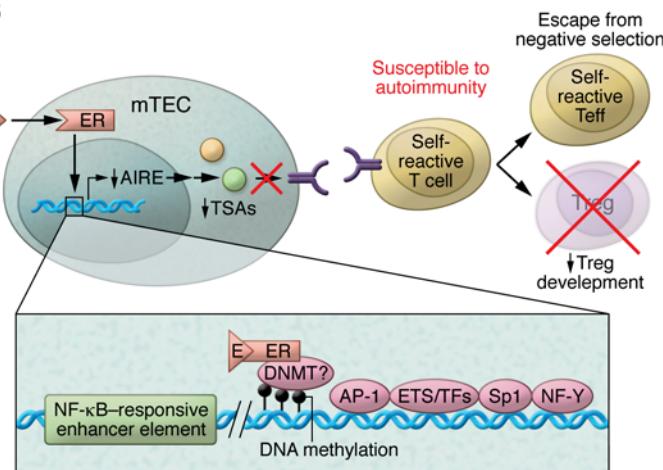
A**B**

Figure 1. Estrogen downregulates AIRE-mediated central (thymic) tolerance. (A) AIRE in mTECs plays a major role in protection against autoimmunity. AIRE upregulates expression of TSAs, so that high-affinity T cells (Teff) that recognize these TSAs are triggered to undergo negative selection. Additionally, AIRE induces Treg development. AIRE expression is regulated by NF-κB responsive enhancer elements (~3 kB upstream of AIRE), transcription factors (Sp1, AP-1, NF-Y, ETS/TFs), and AR [not shown]), and DNA methylation of the AIRE promoter region. (B) Estrogen (E)/ER complexes downregulate AIRE through DNA methylation. Although the mechanism is not known, ER may potentially facilitate DNMT activity or bind to other transcription factors to induce DNA methylation. DNA methylation results in AIRE gene silencing with decreased AIRE-dependent TSA expression, escape of self-reactive T cells from negative selection, and decreased Treg development. These estrogen effects contribute to increased susceptibility to autoimmunity.

tion or that the estrogen/ER complex may indirectly inhibit DNA demethylation. Evidence in support of the former comes from studies in breast cancer that suggest that ER may indeed positively regulate DNMTs (12). It is also possible that estrogen/ER may bind to other transcription factors to enhance transcription. Indeed, ER binding to AP1, which is predicted to bind to the *AIRE* promoter region (13), has been described in other cell types.

Clinical implications of estrogen-mediated AIRE regulation

The finding that estrogen downregulates AIRE has substantial clinical implications. Estrogen downregulation of AIRE may account, in part, for the increased risk of autoimmunity in females. Dragan et al. demonstrated that AIRE expression is lower in females than males, and this difference was only seen after the onset of puberty, a time when females have higher circulating levels of estrogen than males. In postpubertal females, *AIRE* mRNA levels were reduced by approximately 50% compared with those of males (7). This finding is significant in light of recent reports that demonstrated that partial (in addition to complete) loss of AIRE function enhances susceptibility to autoimmune disease. For

instance, patients and mice with a single copy of a dominant *AIRE* mutation have a quantitative decrease in AIRE function and develop autoimmune manifestations (14, 15). Thus, “dialing down” AIRE function to intermediate levels also elevates the incidence of autoimmunity. Given this, the effects of estrogen on reducing AIRE expression could account for the increased autoimmune predisposition noted in females. Estrogen downregulation of AIRE may be particularly relevant to autoimmune thyroiditis, which mostly affects females, as Dragan et al. show that estradiol treatment exacerbates autoantibody production in a mouse model of autoimmune thyroiditis through a thymus-dependent mechanism (7).

Estrogen downregulation of AIRE may be relevant, not only to autoimmunity, but also to cancer. Therapies that block estrogen production or estrogen-mediated effects (such as selective ER modulators [SERMs]) are commonly used to treat breast cancer. These therapies are utilized for their antiproliferative effects on breast cancer cells; however, they may have the unintended consequence of increasing *AIRE* transcription. Since AIRE limits the antitumor immune response against self antigens expressed by tumors (16, 17), increasing *AIRE* transcription may

also stifle an effective immune response against the cancer cells. Thus, therapies that alter estrogen production/effects may have a deleterious effect on the anticancer immune response, a possibility that warrants further study.

Conclusions and future directions

The findings in the study by Dragan and colleagues provoke a number of follow-up questions. First, the authors show that, in addition to estrogen, other sex hormones (progesterone and the androgen dihydrotestosterone [DHT]) also affect *AIRE* mRNA levels. Progesterone, similarly to estrogen, decreased *AIRE* expression, while the androgen DHT increased *AIRE*. What are the mechanisms by which these hormones regulate *AIRE* transcription? Our data indicate that DHT/AR complexes bind directly to androgen response elements (AREs) in the *AIRE* promoter to upregulate *AIRE* transcription (18). Does progesterone act through its receptor to bind directly to the *AIRE* promoter? Or does progesterone function through an epigenetic mechanism?

Second, AIRE is expressed not only in mTECs, but also in extrathymic AIRE-expressing cells (eTACs), thymic B cells, and other cell types (1). How do sex hor-

mones influence AIRE expression in these alternative AIRE-expressing cell types? Furthermore, the transcription factor FEZF2 can regulate the expression of TSAs independently of AIRE (19). Do sex hormones also influence FEZF2 expression? Third, sex hormone profiles are not only different between males and females after puberty, but also in the first six months of life, when neonates undergo “mini-puberty” with adult levels of circulating sex hormones (20). Are differences in AIRE expression already present at this early time period? Testing AIRE expression in this neonatal window is of particular importance, because this time period has been reported to be crucial for AIRE function (21). On the opposite end of the age spectrum, sex hormone production decreases in older adults. What is the effect of decreased sex hormone levels on AIRE expression and autoimmunity risk? A recent study reported that there was no difference in the transcriptome, including *Aire* and AIRE-dependent TSAs, of mTECs from male versus female mice (22), a finding that appears to be in direct conflict with the findings of Dragin et al. A possible explanation for the discrepancy in the two studies is the differences in ages of the mice studied. Dumont-Lagacé and colleagues utilized 24-week-old mice, whereas Dragin et al. evaluated 6- to 8-week-old mice. Of note, thymic involution is considerable in 24-week-old animals; therefore, this additional factor may be important at this time point. Together, both of these reports raise the question of how age, sex hormone levels, and possibly thymic involution might act on AIRE expression in males and females.

Finally, whether sex hormone modulation can be utilized for therapeutic purposes is an intriguing question. In theory, SERMs or other therapies that antagonize the effects of estrogen might be useful for treatment of autoimmune disease. On the other hand, increasing estrogen's

effects, which would lower AIRE expression and allow escape of tumor-reactive T cells from negative selection, might be useful as a cancer immunotherapy. Proof of principle for the latter concept has been established by augmentation of antitumor immunity through transient blockade of AIRE function in adult mice (1). These effects would ideally be limited to mTECs in order to prevent unwanted side effects of sex hormone therapy. Such strategies will require examination in future studies.

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