Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) is a recessive autoimmune disease where patients suffer from endocrine and ectodermal disorders [1]. APECED is caused by a loss of function in the AutoImmune Regulator (AIRE), a transcription factor necessary for ensuring that immune cells do not attack the body, also called tolerance [2]. AIRE primarily functions in the thymus by promoting expression of self-antigens in medullary thymic epithelial cells, which kill T-cells that bind these self-antigens [3]. Recent studies have also identified extra Thymic AIRE expressing Cells (eTACs) in peripheral immune organs, such as lymph nodes and the spleen [4]. Markedly, the self-antigens expressed in thymic cells differ from those in eTACs [4]. *Despite these findings, AIRE’s role in the transcription of unique self-antigens in eTACs remains unclear.*

My **long term goal** is to understand how AIRE contributes to peripheral immune tolerance, which will shed light on why AIRE loss of function causes endocrine and ectodermal disorders in APECED patients. My **primary goal** is to better understand the mechanism by which AIRE promotes the expression of unique self-antigens in eTACs. A mouse model will be used because AIRE knockout mice show similar autoimmune symptoms to APECED patients [5]. My **hypothesis** is that the AIRE transcription complex in eTACs has different coactivators than the thymus, causing the different self-antigen expression between the thymus and periphery.

**Aim 1: Uncover which AIRE domains are sufficient for self-antigen expression in eTACs.** **Approach:** Using mice with single-domain AIRE constructs+GFP and AIRE+GFP wild type (WT) mice, lymph nodes will be collected and GFP+ cells will be isolated using flow cytometry. RNA-seq will be used to quantify the mRNA levels of self-antigens—including hormones, cell adhesion proteins, and mucosal proteins [6]–in WT versus single-domain eTACs. **Rationale:** Examining self-antigen transcripts in WT versus single-domain eTACs will uncover which AIRE domains are sufficient for expressing self-antigens in eTACs. **Hypothesis:** The highest transcriptional expression of self-antigens is expected in WT mice, followed by mice with AIRE’s PHD domain because studies have shown that PHD is an activation domain [7].

**Aim 2: Evaluate whether AIRE mediated self-antigen expression changes over time in eTACs.** **Approach:** After harvesting lymph nodes from AIRE+GFP WT mice at three different age points: young, mature adult, and middle age (1, 5, and 10 months, respectively) [8], and isolating GFP+ cells, RNA-seq will be used to analyze self-antigen mRNA expression in eTACs from each time point. **Rationale:** In humans,initialAPECED symptoms do not present until around age 5, and continue to present throughout life, suggesting that AIRE’s role in eTACs may increase over time. **Hypothesis:** Expression of self-antigens, including hormones, cell adhesion proteins, and mucosal proteins, in eTACs will increase with age.

**Aim 3: Determine which proteins interact with AIRE in eTACs. Approach:** Lymph nodes from WT mice will be collected and Co-Immunoprecipitation will be used to isolate proteins that interact with AIRE, which will then be analyzed using tandem mass spectrometry. **Rationale:** Specific AIRE coactivators are unknown in eTACs, and comparing AIRE coactivators in eTACs to those known in the thymus will enhance our understanding of self-antigen expression in eTACs. **Hypothesis:** Some important thymic coactivators, such as the transcriptional coactivator CBP [9], will interact with AIRE in eTACs, but other coactivators will differ.

Understanding how AIRE promotes unique self-antigen expression in eTACs will shed light on AIRE’s role in maintaining peripheral tolerance. Further research to understand AIRE coactivators in eTACs versus the thymus can uncover the biological significance of these unique self-antigens in eTACs. Eventually, this knowledge gained about peripheral immune tolerance can be applied to treating APECED and other autoimmune diseases.

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