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Many self-reactive T cells are eliminated or become regulatory T cells during their thymic development, while some escape this selection process, for example if they do not encounter their antigen in the thymus. Peripheral lymphoid tissues such as lymph nodes harbor tolerogenic dendritic and stromal cell populations that complement their thymic counterparts in mediating immune tolerance (deletion, anergy or regulatory T cell induction). Most dendritic cell (DC) subsets can either suppress or elicit immune responses depending on perceived signals such as the presence of inflammation or microbial products. This dual function, if deregulated, may contribute to inappropriate activation of self-reactive T cells and autoimmunity. Some subsets, such as the extrathymic AIRE-expressing cells (eTACs) recently identified in mice, appear more stable in their tolerogenic function. The relative tolerogenic potential of different self-antigen-presenting DC subsets in humans and their functional plasticity in the context of inflammation are not understood. Thus, we propose to address this gap under this Exploratory / Developmental R21 grant. Because we were able for the first time to identify and isolate eTACs from the lymph nodes of human donors and humanized mice, we propose as a first objective to establish a novel humanized mouse model using human fetal thymus and fetal liver CD34+ cells in which AIRE-expressing cells will be tracked with GFP driven by the AIRE promoter and sorted as a pure population for transcriptional analysis to identify (1) better surface markers for isolation of eTACs from human tissues, (2) any relationship with other DC populations, (3) specific genes expressed that could play a role in tolerance induction in these cells, and (4) possible tissue-specific self-antigens that they may express. To determine any relationship with other DC populations, we will sort and perform transcriptional analysis on three other well-characterized DC subsets obtained from the lymph nodes of the same humanized mice. As a second objective, we will compare the tolerogenic function of eTACs and other DC populations. We will express, also in fetal liver CD34+ cells, a T cell receptor (TCR) that recognizes a MART-1 peptide presented on HLA-A2. The CD8+ T cells bearing this TCR are not deleted in the human thymus. We will also express the MART-1 antigen along with GFP under the AIRE or DC subset-specific promoter. We will then analyze the outcome (deletion, anergy or activation) of the CD8+ T cells that have encountered their antigen presented by eTACs in lymph nodes or by other DC subsets in secondary lymphoid tissues (and possibly in the thymus). In this system, we will also evaluate whether the function of these APCs, expected to be tolerogenic under steady-state conditions, is affected by inflammatory conditions. This study is important to evaluate how different subsets of human hematopoietic antigen-presenting cells perform their tolerogenic function in vivo and to exploit the most stably tolerogenic populations and/or the biological pathways that they utilize to create better tolerance-inducing therapies for autoimmune diseases.