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Inadequate recognition of autoantigens during thymopoiesis can result in abnormally high frequency of autoreactive T cells, which can lead to autoimmune diseases when combined with defective mechanisms of peripheral tolerance in susceptible individuals. The process of thymic selection of autoreactive T cells in humans is largely not understood due to lack of in vivo models and could so far only be extrapolated from rodent studies. Analysis of autoantigen expression in the human thymus has provided an incomplete picture based on increasing evidence that this does not necessarily correlate with T cell deletion. As autoreactive T cells are also found circulating in 'healthy' individuals, it is unclear to what extent incomplete thymic deletion (or regulatory T cell (Treg) selection) contributes to autoimmunity.

Our proposed research is based on humanized mice reconstituted with fetal hematopoietic stem cells (HSCs) and thymic tissue, whereby HSCs can be transduced to express an autoreactive T cell receptor (TCR) and/or the actual epitope recognized by this TCR. The HSCs then develop into T cells in a normal human thymus in vivo, a fraction of which display the autoreactive TCR. Having established the model with T cells specific for a melanocyte autoantigen that fail to undergo thymic deletion unless the epitope is also introduced in HSCs, we will adapt this system to a collection of TCRs that have been identified in islet infiltrates of Type 1 diabetes patients. These TCRs were selected because it is yet unknown whether their specific epitopes are presented and recognized in a normal human thymus. Thus the model will help determine whether T cells bearing those TCRs escape thymic deletion through a number of mechanisms that will be tested (lack of expression, of crossreactivity or of neoepitope formation). Introduction of the cognate epitopes in HSCs will allow us to evaluate their effect on developing autoreactive T cells (deletion versus positive selection of Tregs), and further explore the unique contribution of specific subsets of antigen-presenting cells (APCs) in this process, including their ability to relocate to the thymus from the bloodstream to influence thymic selection. Using recipient mice that are able to present antigens to human T cells and antigens that are cross-reactive between the two species, we will assess the conditions that are needed to activate autoreactive T cells to attack their target tissue. This work will feature immunofluorescence imaging, multi-parametric flow cytometry and single-cell RNA sequencing analysis to analyze human APC subsets that populate the thymus and peripheral lymphoid tissues and engage human autoreactive T cells, and to assess the phenotype and molecular profile of these autoreactive T cells based on different conditions of antigen encounter (thymus vs periphery, type of APC, etc). Overall, these studies will provide valuable insights into the generation of human autoreactive T cells causing autoimmune diseases, their reactivity to autoantigens in vivo, and will facilitate the development and evaluation of new therapeutic approaches targeting autoreactive T cells for deletion or tolerance.