Quantum Dot MHC Multimers

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Purpose: We recently developed a novel strategy allowing simultaneous detection of multiple islet autoreactive CD8 T-cells through specific binding of their T-cell receptor epitope recognition domain. We wish to relate the islet autoreactivity profile of CD8 T-cells in insulitic lesions with that of other regions in the body of this same pancreas donor (pancreas draining and non-draining lymph nodes, peripheral blood and/or spleen), using this novel methodology.

Methods: This strategy involves a combinatorial quantum dot MHC multimer nanotechnique to simultaneously monitor the presence of HLA-A2 restricted CD8+ T-cells against a wide range of candidate islet autoantigens (insulin, pre-pro-insulin, IA-2, GAD65, IGRP and ppIAPP) that has been validated in recent onset diabetes patients, their siblings, healthy controls and islet cell transplantation recipients. These novel HLA multimer reagents have been scrutinized for their specificity, peptide epitope purity and valency. Using this kit, islet autoreactive CD8+ T-cells recognizing a range of islet autoantigens were shown to be preferentially detectable in recent onset patients, but rarely in healthy controls. Applying this methodology to samples of islet cell transplantation recipients allowed detection of changes of autoreactive T-cell frequencies against multiple islet cell derived epitopes that was associated with disease activity and correlated with clinical outcome. Our novel approach allows simultaneous detection of CD8+ T-cells reactive to multiple HLA-A2-restricted beta cell epitopes requiring limited amounts of blood, without a need for in vitro culture (in contrast the current methods for detection of islet autoreactive T-cells), that is applicable on stored blood samples.

Summary of Results: Using the very same HLA class I tetramers and islet epitopes, we are currently comparing insulitic T-cell specificities with that in other tissues. Since we only need very small aliquots of cryopreserved PBMC, PDLN or spleen cells, our request should supplement other current activities in cellular islet autoimmunity in nPOD. In the context of current nPOD activities at the LIAI (Dr. Von Herrath) we have investigated T-cell autoreactivity in situ in insulitic lesions using the same source of HLA monomers. Preliminary data suggest that mesenteric lymph nodes drain the pancreas as indicated by increased frequencies of T cells to GAD65, in contrast to mice, and therefore seem to disqualify as `control' tissue.

Conclusions: Our technology delivers in nPOD T cell specifities detectable in circulation and lymph nodes can be found in insulitis. We currently define the frequency and specificity of islet autoreactive CD8 T-cells in pancreas draining lymph nodes, control lymph nodes, spleen and/or peripheral blood of larger series of HLA-A2 positive diabetic and prediabetic organ donors and compare islet autoreactivity in periphery with that in insulitic lesions.