2016

Infiltration of Identical T Cell Repertoires in Multiple Organs with Autoimmunity in NOD Mice

Sydney Look, Laurie Landry, Theodore Williams, Thomas Delong, Kathryn Haskins and Maki Nakayama

Infiltration of Identical T Cell Repertoires in Multiple Organs with Autoimmunity in NOD Mice

Sydney Look, Laurie Landry, Theodore Williams, Thomas Delong, Kathryn Haskins, and Maki Nakayama at the Barbara Davis Center for Childhood Diabetes
2016 Child Health Research Summer Internship at the University of Colorado School of Medicine and the Colorado Children's Hospital

Introduction

Patients with one autoimmune disease, such as Type 1 Diabetes, are at a higher risk for developing additional autoimmune disease(s) than healthy individuals. For example, approximately one third of patients with Type 1 Diabetes are additionally diagnosed with another autoimmune disease, such as Hashimoto disease targeting the thyroid, Chrohn's disease targeting the intestine, or Addison's disease targeting the adrenal gland.

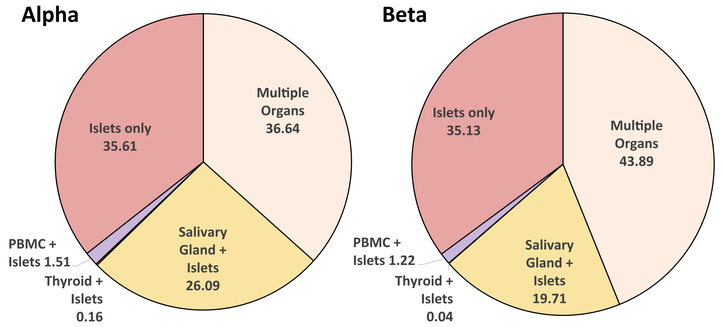
Similarly, non-obese diabetic (NOD) mice spontaneously develop both autoimmune diabetes, affecting insulin production in the pancreas, and Sjögren's syndrome, affecting saliva and tear production in the salivary glands.

In individuals affected by autoimmune diseases, defective T cells in the immune system play a role in the destruction of cells specific to the organ they have infiltrated. In addition to a genetic predisposition to multiple autoimmune diseases, we hypothesized that T cells activated in a primary organ may play a role in the development of secondary autoimmune diseases. If this is true and if we can characterize those T cells infiltrating multiple organs, we may be able to target these T cells in an effort to prevent a secondary autoimmune disease from developing.

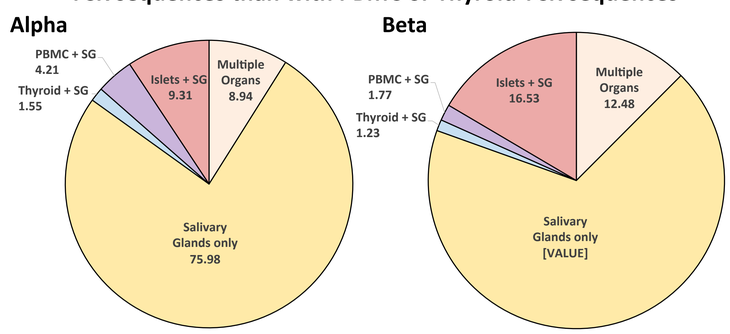
Methods

To determine if and how often identical T cells infiltrate multiple targeted organs and whether the infiltration is antigen-specific, we analyzed T cell receptor (TCR) repertoires in pancreatic islets, salivary glands, thyroid, and peripheral blood of NOD mice, using single cell PCR and bulk sequence analysis to find potentially good TCR candidates present in multiple organs. We then utilized a retroviral expression system in order to make "artificial" T cells expressing identical TCRs. We tested the reactivity of dual-infiltrating TCRs to these tissues using ELISA to measure the amount of Interleukin-2 secreted in order to determine whether or not there was an antigen response.

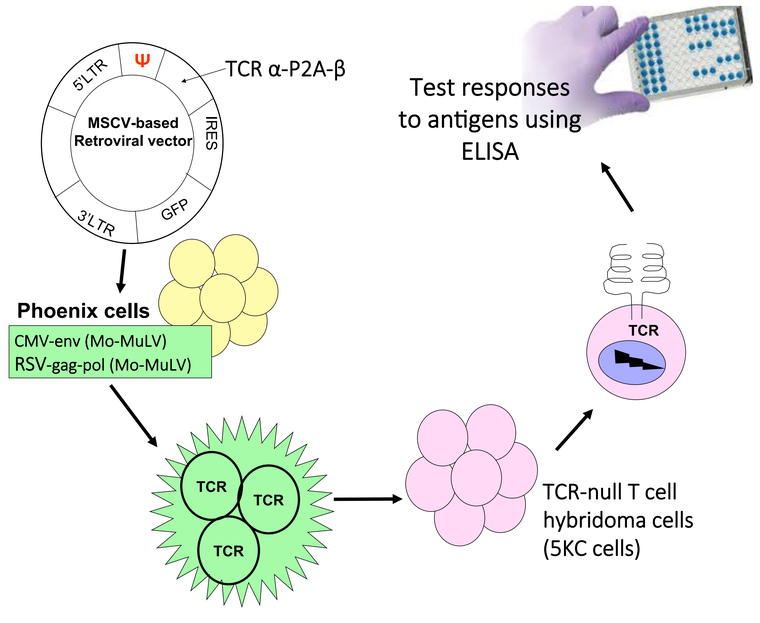
More Islet TCR sequences were overlapping with Salivary Gland TCR sequences than with PBMC or Thyroid TCR sequences



More Salivary Gland TCR sequences were overlapping with Islet TCR sequences than with PBMC or Thyroid TCR sequences



Testing the antigen specificity of TCRs



Results

We found that there is a higher frequency of overlapping TCR sequences between islets and salivary glands than between islets and the other organs (22.9 ± 3.19% with salivary glands vs 0.10 ± 0.06% with thyroid, 1.37 ± 0.15% with peripheral blood). In addition, the single cell TCR analysis identified identical TCR pairs at the nucleotide level including a second alpha/beta sequence in both islets and salivary glands, indicating the presence of T cells expanded from monoclonal T cells in these organs. Six out of 7 TCRs that were found in both islets and salivary glands responded to islets but not to salivary glands.

Antigen Responses of Selected T Cell Receptors using ELISA

	40.A5	40.B4	40.G11-2	41.A6	41.D6	41.G9	48.A4	No TCR Expressed	Known Insulin-Reactive TCR
Insulin Peptide B9-23	None	None	Very Strong	None	None	None	None	None	Very Strong
Chromogranin A Mimotope (ps3)	None	None	None	None	None	None	Very Strong	None	None
Pancreatic Islets	Weak	Weak	Strong	None	Moderate	Strong	Moderate	None	Weak
Salivary Gland	None	None	None	None	None	None	None	None	None
Thyroid	None	None	None	None	None	None	None	None	None
Frequency in Islets (%) (~1600 unique sequences)	1.06 (top 20)	3.15 (top 3)	0.31 (top 50)	*	0.34 (top 50)	*	0.09 (top 150)	* Found in single cell PCR but not in bulk sequence analysis	
Frequency in SG (%) (~3000 unique sequences)	0.30 (top 50)	0.39 (top 50)	0.05 (top 400)	0.03 (top 1000)	0.05 (top 400)	*	*		

None -13 to 10 | Weak 11 to 60 | Moderate 61 to 350 | Strong 351 to 1500 | Very Strong 1501 to 5590

Discussion

In conclusion, we demonstrated identical T cells infiltrating in both pancreatic islets and salivary glands. However, the current study does not support the concept that T cells in an affected organ migrate to another due to antigen specificity.

Future Directions

In order to say whether or not there are dual-reactive TCRs capable of targeting multiple organs, we must first determine without a doubt that our results are true. To do so, we need to test a positive control for the salivary gland. We can both obtain cell lines from the salivary gland and make artificial T cells with frequent TCRs in salivary glands to test with our system. If our results are true, the next step would be to determine if T cells migrating to secondary organs can damage the organ despite a lack of antigen-specificity. If this is true, regulating these TCRs may be able to help prevent development of additional autoimmune diseases.