Nicholas Moehn

**Specific Aims**

The adaptive immune system is the body’s most powerful tool in fighting off infections. In individuals with severe-combined immunodeficiencies (SCIDs), T and B cell production is greatly decreased or absent. The SCID Omenn syndrome causes the low B and T cell counts typically found in SCIDs. The mutation is typically “leaky”, allowing some T and B cells to be produced, but not as many as in a healthy individual. What makes Omenn syndrome unique among SCIDs is that the adaptive immune cells that are produced are autoreactive, causing autoimmune symptoms. The immunodeficiency found in individuals with Omenn syndrome is caused by a mutation in a gene called *RAG1*. *RAG1* encodes the RAG1 protein, which is involved in the production of the receptors that adaptive immune cells use to determine their specific target. AIRE is a transcription factor that activates genes involved in the negative selection of self-reactive immune cells. *How exactly* *RAG1 mutation contributes to the Omenn syndrome phenotype, and whether or not AIRE is involved, remains unclear*.

**We seek to test the hypothesis that a combination of deficiencies in RAG1 and the transcription factor AIRE creates a small oligoclonal population of immune cells that are self-reactive.** Recently, two individuals with Omenn syndrome were found to also have deficiencies in AIRE. The **primary goal** of this research is to demonstrate that RAG1 and AIRE deficiencies produce the phenotype found in patients with Omenn syndrome. The **long term goal** is to identify the cause of the AIRE deficiency in these patients.

**Specific Aim:** To determine if all individuals with Omenn syndrome are deficient for AIRE.

**Approach:** We will use RNA sequencing to look at the transcriptome of individuals confirmed to have Omenn syndrome. We will compare these transcriptomes to those with typical SCID phenotypes, predispositions to autoimmune disease, and normal immune phenotypes.

**Specific Aim:** To demonstrate that deficiencies in RAG1 and AIRE are sufficient to produce an oligoclonal population of self-reactive adaptive immune cells.

**Approach**: We will use the CRISP-Cas9 system to produce populations of mice that are deficient in RAG1, AIRE, and both. The external phenotype of each of these populations will be assessed. T cells and B cells from each will be collected to determine the variety and specificity of their receptors and we will use tandem MS/MS to look at the receptors and determine the sequence and the specificity of the receptor.

**Specific Aim**: To detect if there is a mutation in the AIRE gene or associated regions that would correspond to its deficiency in individuals with Omenn syndrome.

**Approach**: We will use next-generation sequencing methods to sequence individuals with Omenn syndrome. We will look at mutations across the genome that are conserved across these individuals. We will focus on regions in RAG1, AIRE, and the genes for which AIRE starts transcription.

As of yet, no study has been done to confirm that AIRE deficiency causes the autoimmune symptoms present in Omenn syndrome. This research could confirm this link, which could aid in treatment of individuals with the disease. While Omenn syndrome is a rare disease, understanding the mechanism of its function could also advance our understanding of immunodeficiencies and autoimmune diseases individually