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**Specific Aims**

The adaptive immune system is the body’s most powerful tool in fighting off infections. In individuals with severe-combined immunodeficiencies (SCIDs) such as Omenn Syndrome (OS), T and B cell production is greatly decreased or absent. In OS some, but much fewer, T and B cells are produced. OS is unique among SCIDs—adaptive immune cells are autoreactive, causing autoimmune symptoms. OS is associated with a mutation in *RAG1*. RAG1 is involved in the production of the receptors that adaptive immune cells use to determine their specific target. Recently, individuals with OS were found to also have decreased mRNA count for AIRE, a transcription factor that regulates expression of proteins involved in the negative selection of self-reactive immune cells. *How exactly* *RAG1 mutation contributes to the OS 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 population of immune cells that are self-reactive.** The **primary goal** of this research is to demonstrate that RAG1 and AIRE deficiencies produce the phenotype found in patients with OS. The **long term goal** is to identify the cause of the AIRE deficiency in these patients.

**Aim 1**: To determine if individuals with OS have decreased mRNA for AIRE or other genes involved in adaptive immune cell development.

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

**Hypothesis:** We expect that individuals with OS will have transcriptomes that look like a combination of those found in individuals with autoimmune diseases and SCIDs. **Rationale**: Two individuals have been found to have deficiencies in AIRE mRNA. This would determine if that was the sole cause of autoimmune symptoms or if there are multiple origins.

**Aim 2:** To detect if there is a mutation in the AIRE gene or associated regions that would correspond to its deficiency in individuals with OS.

**Approach**: We will use next-generation sequencing methods to sequence individuals with OS. 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 regulates transcription.

**Hypothesis:** We expect to find mutations in the AIRE gene or an associated gene that would cause its decrease in transcription. **Rationale**: Deficiencies in AIRE are likely due to a mutation either in AIRE itself or one of the gene that regulates its expression.

**Aim 3:** 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. 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 similarities between receptors in each class.

**Hypothesis:** We expect that mice with deficiencies in both RAG1 and AIRE will exhibit immune cell counts similar to those of RAG1 deficient mice with receptors that have sequences similar to AIRE mutants. **Rationale:** A combination of inefficient RAG1 function and lack of immune regulation of receptor specificity should result in an OS like phenotype.

Currently, no study has been done to confirm that AIRE deficiency contributes to the autoimmune symptoms present in OS. This research could aid in treatment of individuals with the disease. While OS is a rare disease, understanding the mechanism of its function could also advance our understanding of immunodeficiencies and autoimmune diseases.