**Specific Aims**

The immune system is a necessary and complex aspect of the human body, but when it fails to function properly the results affect the patient’s quality of life. Allergic rhinitis is a common immune affliction described by symptoms that include sinus swelling, pressure, and sneezing.Allergic rhinitis is caused by a hypersensitivity to antigens in the nasal mucosa. The antigen interaction causes the release of cytokines, including the transforming growth factor beta isoform 1 (TGFB1). In allergic rhinitis patients, TGFB1 is overexpressed [1], ultimately leading to inflammation [2]. Losartan, an FDA-approved drug for high blood pressure, leads to a decreased amount of secreted TGFB1 [3]. However, it is unclear whether Losartan has the ability to treat inflammation associated with allergic rhinitis.

The **overall goal** is to determine if Losartan can decrease the inflammatory symptoms associated with allergic rhinitis. This is based on the fact that allergic rhinitis is an inflammatory disease, and Losartan can decrease the levels and the inflammatory effects of TGFB1. This knowledge will apply to the **long-term goal** of the research, which is to therapeutically control the levels of TGFB1, eliminating the allergic rhinitis response. *Danio rerio* will serve as a model organism due to their usefulness in testing anti-inflammatory drugs and the transgenic model of neutrophilic inflammation, which expresses GFP under a neutrophil specific marker. [4] Polysorbate 80 with trace amounts of hydrogen peroxide can be used to induce an allergic response in the fish. [5]

**Aim1: Determine conserved amino acids in TGFB1 that are important for inflammatory function.**

**Approach:** Use NCBI Blast to identify homologs of the TGFB1 gene in organisms with a diversity of immune systems, then align amino acids using Clustal Omega and identify conserved regions among similar immune systems. Then use CRISPR to knock out these sequences, introduce the allergen, and assay for inflammatory response.

**Hypothesis:** Organisms with an adaptive immune system will share highly conserved regions of the protein, and organisms with only an innate immune system will have altered regions or deletions in place of these conserved acids.

**Rationale:** Understanding the conserved regions will help control for the slight differences in the TGFB1 protein between zebra fish and humans. The conservation of the immune function of the protein will ensure that Losartan’s efficacy results are not simply an error based on any unforeseen differences in the protein and pathway.

**Aim 2. Determine changes in overall gene expression with and without Losartan treatment in an allergic zebra fish model.**

**Approach:** Create four tanks of animals: wildtype (wt), allergic, wt+Losartan, and allergic+Losartan. Allergic response will be induced with polysorbate 80 and hydrogen peroxide. Set tanks for 24 hours, then retrieve tissue from each group of fish, purify the mRNA and sequence the transcriptome. Visualize the data using a heat map including genes with 2 fold or more differences in expression from the wild type expression.

**Hypothesis:** Gene ontology (GO) categories such as defense response will be upregulated in the allergic fish without treatment. Wt+Losartan, wt, and allergic+Losartan will have similar regulation in inflammatory ontology categories. Losartan groups may also experience downregulation in genes associated with maintenance of blood pressure.

**Rationale:** Confirming that genes, like TGFB1, which deal with immunity and allergy are in fact downregulated in the presence of Losartan will provide knowledge about its efficacy in treating allergic rhinitis symptoms. In addition, sequencing may offer insight into possible side effects of the drug and what precautions should be taken.

**Aim 3. Quantify protein levels of TGFB1 in the presence and absence of Losartan.**

**Approach:** Populations will be maintained as in Aim 2.Stable Isotope labeling in fish food with Lys-6 and reverse liquid chromatography will be used to obtain mass spectrometry data on the quantities of gene ontology categories of proteins. Western blotting for protein levels will serve as a confirmation of this quantification.

**Hypothesis:** GO categories like defense response will be large in the allergic model, and the quantities of TGFB1 will be high. In wt, wt+Losartan, and allergic+Losartan, the quantities of TGFB1 will be much lower, and the GO categories will mimic wt. I also expect decreases in blood pressure maintaining proteins for both Losartan groups.

**Rationale:** Quantifying protein levels will solidify Losartan’s ability to mediate the immune response in allergic rhinitis. Gene ontology categories of protein levels allow for a broader picture on Losartan’s function. In addition, quantification of protein levels allows for statistical analysis, and concrete evidence of Losartan’s efficacy.

Defining the regulatory effects of Losartan on the overexpression of TGFB1 brings us one step closer to the successful treatment of allergic rhinitis. Understanding Losartan’s role in regulating proteins associated with allergy can illuminate the usefulness of the drug as an allergy treatment. The key to allergic rhinitis symptoms lies in TGFB1, and treatment with Losartan could be the answer to the widespread disease that plagues millions.

**References**

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