The Effect of Overexpression of IRF5 on B-Cell Inflammatory and Co-Stimulatory Activity

Brigham Young University Honors Thesis Proposal

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Project Purpose

The purpose of this project is to understand the effect of IRF5 overexpression on B-cell on inflammatory and costimulatory activity.

We hypothesize that the overexpression of IRF5 will cause an increase in production of the inflammatory cytokines, IFN-α, IFN-γ, IL-6, IL-4 and a decrease in the anti-inflammatory cytokine, IL-10. This project will examine how the Systemic Lupus Erythematosis (SLE) risk allele, IRF5, affects B cells and their involvement in SLE. We predict that in addition to antibody production, B-cells are important contributors in the activation of other cells that contribute to the pro-inflammatory condition of SLE.

Project Background

Over 5 million people in the world are impacted by one of the four forms of lupus, including an estimated 1.5 million Americans, with 70% of these cases being SLE (1). It is estimated that the incidence rate of lupus is approximately 16,000 new cases per year (1). Although people from any age group can be affected by lupus, women of child-bearing years between the ages of 15-44 are the most at-risk gender and age group (1). According to the Centers for Disease Control and

Prevention (CDC) minority and ethnic groups are affected more than Caucasians—this includes blacks/African Americans, Hispanics/Latinos, American Indians/Alaska Natives, and Asians (1).

SLE is a chronic autoimmune disease characterized by inflammatory tissue damage inflicted by the body's immune system (2). The unregulated activation of the immune system often leads to an overproduction of autoantibodies causing a deposition of immune complexes (3). The inflammatory response then becomes excessive due to unchecked release of cytokines (3). SLE patients regularly show abnormal levels of cytokines (3).

Failure to restrict the hyperactivation of the immune system of a lupus patient can result in severe physiological damage or even, in some cases, death (2). The effects of lupus differ from case to case, from extreme tissue damage to no visible symptoms at all (1). SLE can affect various organs and systems of the body including the skin, joints, brain, lungs, kidneys, and blood vessels—the effects on the targeted organ can lead to pervasive inflammation (2). Although treatment is available for the disease, and correlations to pathway precursors as well as risk susceptibilities are currently being isolated, the pathogenesis of SLE nonetheless remains unclear (3).

The heterogenous nature of SLE, including the complexity of the genetics and the various symptomatic expressions that are often easily misdiagnosed, makes the pathogenesis of this particular autoimmune disease elusive (4). Although the causes of SLE are still unknown, researchers suspect the disease to be associated with environmental, genetic, and hormonal factors (2). These factors seem to work together in the creation of pathogenesis, particularly for

individuals with a disease-prone genetic background. Our research delves into some of the molecular and cellular functions of the suspected genetic factors of SLE, particularly into the functions of the interferon regulatory factor 5 (IRF5) on B-cells and cytokine signaling. IRF5 is known to have strong correlation with increased risk of SLE development affecting B-cells (4). This project will help us better understand the correlation between IRF5, a known risk haplotype for lupus, and the symptoms of lupus.

IRF5, a protein encoded by the IRF5-gene, not only anchors the immune response to pathogens, but is also critical in the regulation of genes responsible for apoptosis, the cell cycle, cell adhesion, all of which are processes highly influential in initiating autoimmunity (5). Patients with SLE often have high levels of type I interferon (IFN) in their serum with increased induction of gene transcripts associated with IFNα. (5) IFN and other cytokines have been found to rely upon the functions of several factors, which includes IRF5. (5) High levels of IFN is correlated with disease activity and severity, but whether this relationship is due to a link between genetic variation and pathogenesis, or is simply an expression of an overactive immune system is unknown. (5) By studying the effect of the overexpression of IRF5, we can understand it's role on cytokine expression and the pathogenesis of SLE.

Our search to understanding the inflammatory and costimulatory response of B-cells to the IRF5 gene will allow us to further determine a potential cause of autoimmunity, propelling us to better understanding the mechanism associated with this disease.

Project Profile Body

Activated B-cells internalize and then present specific soluble antigens to T cells, and contribute to the immune response by producing proinflammatory cytokines. We hypothesize that the upregulation of IRF5 will promote B-cell costimulatory activity and inflammatory cytokine production in addition to antibody generation.

We will isolate naïve B-cells from volunteers and transfect them with an IRF5-overexpressing lentiviral vector (IRF5-pULTRA) or a control vector (pULTRA). We will use an IRF5 expression vector under the control of the CD19 promoter. The CD19 promotor enables cloned gene expression in B-cells. This is notable, especially due to our plan to double IRF5 expression levels. Quantification of IRF5 expression will be accomplished by using real-time PCR.

To ensure only transfected cells are compared, transfected B-cells will be identified through their expression of the dTomato fluorescent protein. We will then treat our transfected B-cells with 15 μg/ml anti-IgM, activating the B-cells through BCR leading to calcium flux. This will allow us to measure the B-cell activation response using single cell ratio-imaging to measure intensity and duration of calcium responses.

Using an imiquimod treatment to stimulate TLR7, B-cells will then be analyzed using flow cytometry to quantify the levels of the costimulatory proteins CD80 and CD86—which are expressed upon activation. Comparison of non-stimulated cells, anti-IgM-stimulated cells, imiquimod-stimulated cells, and cells treated with both imiquimod and anti-IgM will be recorded. Each test condition will be compared to the IRF5 transfected control cells.

If the overproduction of IRF5 follows our predictions, we will be able to see an increase in the levels of CD80 and/or CD86—showing us that overproduction or expression of IRF5 leads to increased levels of costimulatory molecules. ELISA will be used to test for the presence of proinflammatory cytokines (IFN-α, IL-6, IL-10, IL-4, and IFN-γ). The concentration of the cytokines will be tested using the cell culture supernatant for the co-stimulation experiment.

These experiments measure multiple parameters, and because of this we will determine the statistical significances between the groups using ANOVA tests R and SPSS. Our lab is proficient in both programs. Each test will be repeated 5 times for adequate data.

Through this experiment, we expect to see an increase in the proinflammatory cytokines, (IFN-α, IFN-γ, IL-6, and IL-4) and a decrease in anti-inflammatory cytokine, IL-10. This data will allow us to see how the SLE risk allele, IRF5, affects B-cell costimulatory activity proinflammatory cytokine production which are important parameters of autoimmune disease.

Anticipated Academic Outcome

The findings associated with this research will be summarized and submitted in peer-reviewed journals, and will be presented at the American Association of Immunologists and American College of Rheumatology meetings.

Qualifications

I am a junior majoring in Physiology and Developmental Biology, and have worked in Dr. Poole's lab since the fall semester of 2015. We have previously examined the relationship

between the EBV induced gene, EBI-3 and its effect on B-cell migration. I continue to study, learn, and gain familiarity with autoimmunity research.

Dr. Brian Poole has published many papers on autoimmunity, with an emphasis on lupus. He has mentored numerous undergraduate students as a professor at BYU. Dr. Poole possesses the ability to explain complex molecular mechanisms in a simple, and understandable manner, allowing his students to be confident and comfortable in the lab environment. He will be able to help me successfully complete this project.

Dr. Jeff Edwards has had extensive experience in research and teaching in physiology and cell and molecular biology. He has taught various courses to undergraduate and graduate students at BYU, and has mentored numerous students in undergraduate and graduate research. As the PDBIO Honors Coordinator, he understands what is necessary for a successful honors thesis and will help me as I work on this honors project.

Dr. Scott Weber studies molecular immunology and understands the process necessary for successful undergraduate research. His studies focus on the mechanisms of T cells in the immune system. He is also expert in measuring calcium levels, and when the time comes to do these measurements, he will be able to ensure that I complete this technique accurately. His familiarity with immunology will give him the background necessary to answer my questions and further my understanding about my research.

Project Timetable

We will complete the project within 5 months.

May-June: isolate, transfect, and ensure transfection of naïve B-cells.

June-July: treat with anti-IgM and calcium flux measurement

July-August: flow cytometry and incubation and ELISA

August-September: compile and analyze data

September-October: write summary and conclusion

Thesis Defense ?

References

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Honors Timeline

August-September: Project completion, data compilation complete

August-December: Thesis draft writing, defense and submission preparation

December-January: Schedule thesis defense, and submit thesis defense information form (Personal deadline: before January 1, office deadline: February 9)

January-February: Thesis Defense, and thesis Submission Form (office deadline: March 9)

February-March: Finalize and upload thesis (Personal Deadline--office deadline: March 16)