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Effects of FTY-720 on a murine model of Sjogren's syndrome

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Abstract

Proposal and Goals:

My current research interests include *in vivo* Sjogren's syndrome pathology studies in the AEC mouse model as well as *in vitro* auto-antibody and macrophage study. My *in vitro* work looks specifically at whether or not certain auto-antibodies induce phenotypic alterations in macrophages. Specifically, I am examining cell surface markers and cytokine production. I hope that my research findings will provide a deeper insight into and understanding of the basic science behind autoimmune diseases.

There are two goals:

1. To gain familiarity with important techniques in immunology (flow cytometry, immunohistology, animal handling, and others).
2. To learn about human Sjogren's syndrome and animal models, and will become acquainted with the current thinking about pathogenesis of this illness. It is expected that, by the end of the funded period, she will have generated preliminary data leading to further work in this area, and will have learned about interpretation of experimental data.

Project

It is thought that Sjogren's syndrome (SS) progresses in a series of phases. First, before visible signs of autoimmunity, irregular physiological and biochemical events occur. These, perhaps genetically based, events include delayed salivary gland organogenesis as well as increased acinar cell apoptosis. Then, macrophages and dendritic cells are attracted to the exocrine gland as a result of this tissue injury. The macrophages and dendritic cells recruit T and B cells; these cells form lymphocytic foci (LF), some of which histologically appear as germinal centers. Finally, clinical disease is detectable; SS is most often defined by salivary and lacrimal gland secretory dysfunction. This dysfunction most likely result from both the production of autoantibodies that interfere with the neural-acinar cell signaling pathways and advancing loss of acinar cell mass (result of effector T cell action).

In our proposed study, we will use the mouse strain C57BL/6.NOD-Aec1Aec2 as our experimental SS model. It has been found that the introduction of specific Idd (insulin dependent diabetes) genes from the NOD mouse into C57BL/6 background mice lead to the development of SS-like disease in these C57BL/6 mice. These genetic regions are Idd3 (Aec1) on chromosome 3 of the NOD mouse and Idd5 (Aec2) on chromosome 1. Although various mouse strains exist which provide excellent models for the study of autoimmune disorders, only in the AEC strain has decreased salivary flow been seen; therefore, it provides the best model for SS study.

With these mice, we will administer a novel immunosuppressant called Oral Fingolimod

(FTY720, Novartis Pharma) and look at its effect on disease state. This drug is currently in phase 3 studies involving patients with relapsing–remitting multiple sclerosis; results seem promising. FTY is a product of ascomycete *Isaria isinclarii* and resembles sphingosine, an 18-carbon amino alcohol with an unsaturated hydrocarbon chain. Once FTY720 is ingested, its homology to sphingosine allows sphingosine kinases to rapidly phosphorylate the drug. Sphingosine-1-phosphate (S1P), as well as its mimetic FTY-P, have the ability to bind a set of four cell surface G-protein coupled receptors (S1PR1,3,4,5). Three of these receptors (S1PR1,4,5) are found on lymphocytes, and it has been discovered that S1PR1 plays a critical role in lymphocyte egression from 2o lymphoid organs (spleen, lymph nodes) to blood. S1P or FTY-P interaction with cells causes immediate internalization of the S1P1 receptor and an inability of cells to egress from secondary lymphoid tissues. Since SS appears to result from inappropriate homing of lymphocytic cells to exocrine glands, we suspect that, by treating with FTY720, we may prevent these cells from traveling through the blood system to the glands and reduce disease severity. Therefore, we will look at the effect that FTY treatment will have on SS prone mice.

Specific Aims

1. Determine the degree to which treatment with oral fingolimod (FTY720) at 0.3mg/g can suppress lymphocyte circulation in C57BL/6.NOD-Aec1Aec2 mice.

Our unpublished work and the work of others , have established that a treatment dose of 0.3mg FTY720 per gram of mouse three (3) times weekly is sufficient to sequester lymphocytes in the secondary lymphoid organs of C57BL/6 mice without detrimental side effects. In this study, we will confirm these previous findings regarding the immunosuppressive nature of oral fingolimod.

2. Determine whether or not treatment with FTY720 affects lymphocytic infiltration of salivary glands in C57BL/6.NOD-Aec1Aec2 mice.

Like SS patients, animals exhibiting autoimmune exocrinopathy (AEC) have lymphocytic infiltration of the salivary glands, with the majority of these lymphocytes being CD4+ T-cells. These infiltrates appear as peri-ductal and peri-vascular foci within the glandular architecture of the salivary gland. Since FTY720 sequesters lymphocytes in secondary lymphoid organs, we expect to find an overall decrease in the number of infiltrating lymphocytes and hence a decrease in the number and size of peri-ductal and peri-vascular foci.

3. Determine the effect of treatment with FTY720 on salivary flow rate in C57BL/6.NOD-Aec1Aec2 mice.

Since FTY720 sequesters lymphocytes in secondary lymphoid organs, we expect there to be fewer infiltrating T and B lymphocytes in the salivary glands of treated mice, less tissue destruction, and perhaps improved salivary flow.