

James Rogér, DDS and PhD candidate

University of Rochester School of Medicine and Dentistry

“Autoantibody characterization in salivary gland B cells in primary Sjögren’s syndrome”

Abstract:

This project will test the hypothesis that B cells infiltrating the salivary glands in Sjögren’s syndrome (SS) are enriched in (and selected on the basis of) autoreactivity against disease-specific salivary antigens. I will interrogate the infiltrating memory B cells in order to understand their antigenic reactivity. I will take advantage of new methods for the development of human monoclonal antibodies (EBV immortalization of CpGDNA-stimulated memory cells and single-cell PCT) to characterize the antigenic reactivity of the memory B cell repertoire expanded in the salivary glands of SS. Monoclonal antibodies will be produced, and these antibodies will then be tested for reactivity against conventional SS autoantigens (Ro, La, RF or alpha-fodrin). Using immunofluorescence, antibodies will be screened for reactivity against salivary epithelial cells.

The information derived from this limited aim will also allow us to compare the antibody repertoire in the salivary glands with the one expressed in the peripheral blood and saliva. The specific experiments presented in section 3 will be conducted as a student summer research rotation in conjunction with further aims throughout a larger projects that will allow us to better understand the pathogenic process resulting in the salivary infiltration by autoimmune memory B-cells and to develop a practical approach for the measurement of autoantibodies as SS biomarkers in the clinic. This rotation is designed to provide the experience necessary for me to decide if immunology, Sjögren’s syndrome and autoimmune disease will be the focus of my formal PhD thesis work in subsequent years.

The Sanz laboratory has developed expertise in the use of CpG DNA for the stimulation of memory B cells, an assay of central importance for the experiments proposed in this project. We have used CpG DNA to induce proliferation of human memory cells and promote their differentiation into antibody-secreting plasma cells, the assay of which represents the basis for the immortalization of infiltrating memory B cells.

Salivary gland B cells will be purified and memory B cells sorted as established in the Sanz laboratory for previous study of lupus B cells in tonsil biopsies. I will take advantage of a newly developed method that greatly increases the efficiency of immortalization of human memory B cells. In this approach, sorted memory B cells are immortalized with EBV in the presence of irradiated mononuclear cells and CpG DNA oligonucleotide (Cpg 2006). If this method does not prove effective, a single-cell PCT approach will be

employed as an alternative. After two weeks, the culture supernatants will be tested for antibody production. Antibody-producing cultures will be expanded to multi-well plates, at which point the supernatants will be screened for autoantibody activity, and the cell lines will be frozen in liquid nitrogen.