Discovery and Characterization of Antibody Biomarkers of Autoimmune Disease and Reagents for Their Detection

Serra E. Elliott and Patrick S. Daugherty
Department of Chemical Engineering, University of California at Santa Barbara, Santa Barbara, CA 93106-5080
Tel: 805-893-3477 Email: psd@engineering.ucsb.edu

Summary: Using bacteria display, we have isolated disease specific peptides and further analyzed a previously determined autoantibody epitope in a pregnancy related disease, pre-eclampsia. Characterizing these peptides and their binding motifs can not only lead to improved diagnostics but a better understanding of disease pathogenesis.

Bacteria-displayed peptide libraries paired with Fluorescence Activated Cell Sorting (FACS) provides a method to quantitatively screen for small binding ligands to a variety of protein targets. By using an antibody repertoire isolated from diseased patients, we seek to utilize bacteria display to discover and characterize peptides specific to a diseased state [1]. The discovery of these unique peptides can lead to establishment of biomarkers for improved diagnosis and understanding of disease pathogenesis in autoimmune diseases. As testing grounds for methodology development, we will focus on the development of molecular diagnostics for pre-eclampsia, a disease that affects approximately 5% of pregnancies [2], but remains poorly understood. In pre-eclampsia, previous studies have reported that patients develop autoantibodies against the angiotensin II AT1 receptor [3]. These antibodies, upon injection into pregnant mice, induce the symptoms of pre-eclampsia [2]. Based upon these findings, we have expressed the seven amino acid epitope on the surface of \textit{E. coli} for further analysis with a new patient cohort of both pre-eclamptic and “normal” pregnant women. Additionally, we screened a bacteria-displayed peptide library with this patient set (Figure 1). The isolated peptide mimics (mimotopes [4]) were grouped according to common motifs, and nine unique mimotopes have demonstrated significantly higher reactivity with pre-eclampsia patients’ samples than “normal” pregnancies. Not only can these peptide reagents be developed into a novel diagnostic array for pre-eclampsia, but the methods developed in this work can also be generally applied to diagnostic development for autoimmune diseases for which clear biomarkers remain to be discovered.

Fig. 1: The above schematic outlines the screening strategy utilized to isolate disease specific peptide binders. Two groups of disease IgG are labeled red and green, while “normal” IgG is left unlabeled. These are all incubated with non-library expressing bacteria to remove bacteria-specific IgG. These are then allowed to incubate with the library maintaining the control IgG in excess to compete with the labeled disease IgG. Sorting is performed to look for binding to both red and green and this is repeated to enrich the library for binders. Finally sequencing and specificity analysis can be performed on. Figure adapted from [1].