Manatee C-Reactive Protein

Florida manatee health is defined based on complete blood cell counts, plasma biochemistry, and determination of serum amyloid A (SAA) levels in plasma. SAA has become an important biomarker of inflammation, increasing to over 500 mg/L in diseased manatees or in manatees injured by boats, however, SAA shows poor correlation with white blood cell counts, suggesting it may offer modest reliability for early stages of inflammation. C-reactive protein (CRP) on the other hand is an excellent biomarker of early stages of inflammation in other mammals, such as humans and dogs. It has not been used as a biomarker for manatees, as the main commercial antibodies prepared for humans do not cross react. Examination of the amino acid sequence of manatee CRP with other mammalian CRPs show divergence in sequence in the N-terminal end of the protein, which is likely the antigenic site. In preliminary data we have used synthetic peptides to the N-terminus of manatee CRP to create polyclonal antibodies in rabbits that may be useful for detecting early inflammation in manatees. The main goal of this project is to test anti-CRP antibodies using plasma of Florida manatees to determine how well they work and how well they correlate with SAA, the currently used diagnostic, to define early stages of inflammation or impaired immune response. **The working hypothesis is that the manatee-specific CRP antibodies will be more sensitive than SAA at identifying inflammation in manatees.** We have access to manatee plasma samples that we can test with these antibodies for which we have blood cell counts and data on their health status. However, before we do a large-scale scan with these antibodies, we must first validate that they recognize manatee CRP in plasma and develop a sensitive diagnostic assay. This project is a collaborative project with Dr. Maite De Maria (USGS). The student would be involved in developing the assay with the following steps.

1. **Characterization of antibodies.** The antibodies will be characterized to determine their type, IgG or IgM using secondary antibodies that are specific to these types using a western blot. The student will learn how to separate the antibodies by gel electrophoresis and transfer to a membrane and perform a western blot with specific diagnostic antibodies against antibody class.
2. **Proteomics identification of the antigen recognized by the antibodies.** The student will perform western blots on manatee plasma proteins and determine which band is recognized by the antibodies. The band in the gel will be excised and provided to the proteomics core for their identification of the protein. The student may also perform pull down assays from plasma to identify proteins that are bound to the antibodies. CRP has a mass of about 200K daltons, so it should be relatively easy to identify the positive band by gel electrophoresis.
3. **Affinity purification of CRP antibodies**. We will construct an affinity column using the peptide that was used to create the antibodies in rabbits. The student will pass the antibody preparation through the column. Specific antibodies to the peptide will stick to the column and will be eluted with a low pH wash. Immediately upon elution, the affinity purified antibody fraction will be buffered back to neutral and will be tested by ELISA with manatee samples.
4. **Develop specific ELISA for manatees**. Plasma samples of several manatees will be tested with the specific affinity purified antibodies and compared to results from crude antibody preparations to determine the best way forward to measure manatee CRP.
5. **Manuscript**. The student will write a short manuscript detailing the purification and ELISA assay development. We will test a few manatee samples to validate the assay.