

NDnano Undergraduate Research Fellowship (NURF) 2015 Project Summary

1. Student name: Maura Vrabel
2. Faculty mentor name: Dr. Basar Bilgicer
3. Project title: Selective Inhibition of Allergic Responses and Autoimmunity
4. Briefly describe any new skills you acquired during your summer research: I learned how to make liposomes using an extruder and how to synthesize peptides using solid phase Fmoc chemistry. In addition, I learned how to purify these peptides using High Performance Liquid Chromatography (HPLC).
5. Briefly share a practical application/end use of your research: The end goal of our research is to develop a drug that prevents the cellular process of degranulation which leads to severe allergic responses such as anaphylaxis. Currently, we are working on peanut allergy and are determining which epitopes of the peanut protein Ara h2 are the most potent.

Type I hypersensitivities are the most dangerous of the types of allergy because a limited exposure to the allergen can trigger severe allergic responses, such as anaphylaxis. Anaphylaxis is caused by the release of histamine from the mast cells during the process of degranulation and results in the swelling of the throat and sudden drop in blood pressure. Type I hypersensitivities includes severe food allergies, which are difficult to control because of the tight binding of the Immunoglobulin E (IgE), a type of antibody, to the FcεRI receptors on the surface of mast cells. Once bound, the IgE will not unbind from the receptor and the two are considered one complex. The clustering of these IgE-FcεRI complexes is the cause of degranulation (Figure 1). The only way to prevent allergic reaction is complete avoidance of the allergen. The current medical solution for combatting these life-threatening reactions is the use of epinephrine auto-injectors, such as EpiPen. However, this method only slows down the reaction long enough for the person to receive professional medical care. Severe allergy sufferers must always carry the auto-injector on their person in case of an emergency and must follow a restricted diet. There is no preventative cure. The overarching goal of my project is to develop a drug to inhibit the cellular response of degranulation, so as to prevent the release of histamine in the first place. We have decided to focus on peanut allergy because of its wide occurrence and severity.

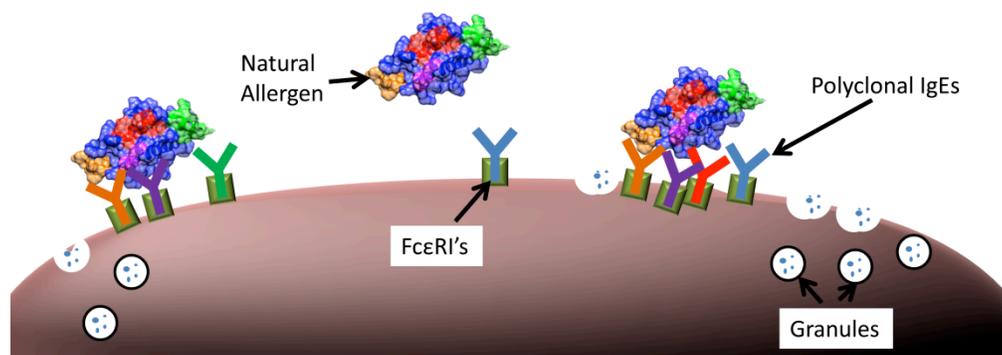


Figure 1. Polyclonal IgEs, attached to the mast cell via FcεRI receptors, bind to the allergen protein which clusters the IgE- FcεRI complexes and produces cross-linking events between the fabs of neighboring antibodies. This cross-linking stimulates degranulation.

Using solid phase peptide synthesis with Fmoc chemistry, I made two inhibitor molecules; one for 2,4 dinitrophenol (DNP) and one for dansyl chloride (Dansyl). DNP and Dansyl are small hapten molecules that have been shown to stimulate degranulation in rat basophil leukemia (RBL) cells, which act like mast cells when primed with the anti-DNP and anti-Dansyl IgEs. By demonstrating successful inhibition of these model molecules, we can apply that knowledge to inhibiting degranulation in vivo. I performed an inhibitor study with the molecules I synthesized and purified over a 3-hr and 24-hr incubation periods, during which the inhibitor molecules were allowed to attach to the IgEs. Figure 2 depicts the results of the 24-hr period where percent degranulation clearly drops as the concentration of the inhibitor molecule increases.

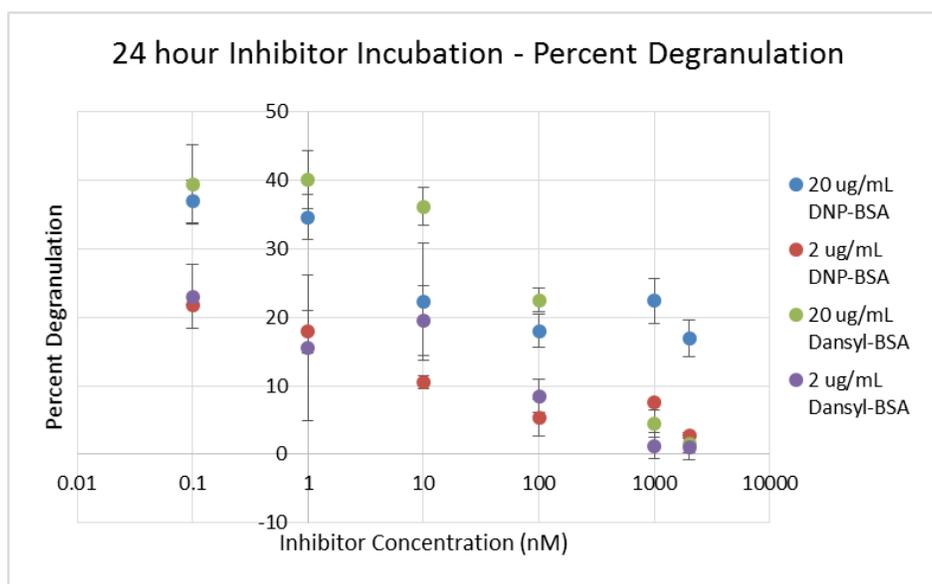


Figure 2. The degranulation of RBL cells using DNP-BSA and Dansyl-BSA allergens at two concentrations shows the efficacy of the inhibitor molecules, reducing the percent degranulation as the inhibitor concentration increases.

Publications (papers/posters/presentations): Nanoallergens: A Multivalent Platform for Studying and Evaluating Allergen Epitope Presentation and Characterization (in preparation)