NDnano
Center for Nano Science and Technology

NDnano Summer Undergraduate Research 2017 Project Summary

1. Student name & university: Joseph Riehm, University of Notre Dame

2. ND faculty name & department: Basar Bilgicer, Chemical and Biomolecular Engineering

3. Project title: Recruitment of Endogenous Antibodies

4. Briefly describe new skills you acquired during your summer research:

During this summer, I have gained experience utilizing different analytical techniques including Spectrophotometry, Dynamic Light Scattering, Enzyme Linked Immunoabsorbance Assay (ELISA), and High-Performance Liquid Chromatography (HPLC). I have also obtained skills and experience in researching, planning, and executing new syntheses and experiments.

5. Briefly share a practical application/end use of your research:

This research has possible applications for a wide spectrum of diseases. One possible use is to recruit antibodies already present in a patient's body to target tumor cells or disease causing agents, such as bacteria or viruses, for destruction by the immune system. For example, the binding of antibodies to a cancer cell may cause destruction of the cell through the antibody-dependent cell-mediated cytotoxicity pathway of the immune system.

6. 50- to 75-word abstract of your project:

The molecule under development is a proof of concept for a peptide that recruits antibodies naturally present in humans to a chosen cell receptor, marking the cell for destruction by the immune system. A series of molecules were designed to multivalently target, thus promoting a covalent conjugation to an antibody and, with a second targeting moiety, attach to another antibody or cell receptor. This complex is designed to securely mark the cell for destruction.

7. References for papers, posters, or presentations of your research:

1. Parker CG, Domaoal RA, Anderson KS, Spiegel DA. An antibody-recruiting small molecule that targets HIV gp120. Journal of the American Chemical Society. 2009;131:16392-16394. doi: 10.1021/ja9057647.

2. Carlson CB, Mowery P, Owen RM, Dykhuizen EC, Kiessling LL. Selective tumor cell targeting using low-affinity, multivalent interactions. ACS Chemical Biology. 2007;2:119-127. doi: 10.1021/cb6003788.

3. Handlogten MW, Serezani AP, Sinn AL, Pollok KE, Kaplan MH, Bilgicer B. A heterobivalent ligand inhibits mast cell degranulation via selective inhibition of allergen-IgE interactions *In Vivo*. J Immunol. 2014;192:2035-2041. doi: 10.4049/jimmunol.1301371.

4. Hasegawa J., inventor; 1980 May 6, O-Hemi-succinate of propranolol. United States patent US 4,201,866 A.

5. Murelli RP, Zhang AX, Michel J, Jorgensen WL, Spiegel DA. Chemical control over immune recognition: A class of antibody-recruiting small molecules that target prostate cancer. Journal of the American Chemical Society. 2009;131:17090-17092. doi: 10.1021/ja906844e.

6. Jakobsche CE, Parker CG, Tao RN, Kolesnikova MD, Douglass EF, Spiegel DA. Exploring binding and effector functions of natural human antibodies using synthetic immunomodulators. *ACS chemical biology*. 2013;8(11):10.1021/cb4004942. doi:10.1021/cb4004942.

One-page project summary that describes problem, project goal and your activities / results:

Antibody therapy has shown great promise for many different applications including cancer, bacteria, and virus therapeutics. These treatments, however, suffer from multiple drawbacks including that antibodies can be extremely expensive, have poor oral bioavailability, and can have serious side effects.[1] One approach, depicted in figure 1, to avoid these obstacles is the use of a small molecule to recruit endogenous antibodies, which are antibodies already present in an individual's bloodstream, instead of introducing external antibodies.

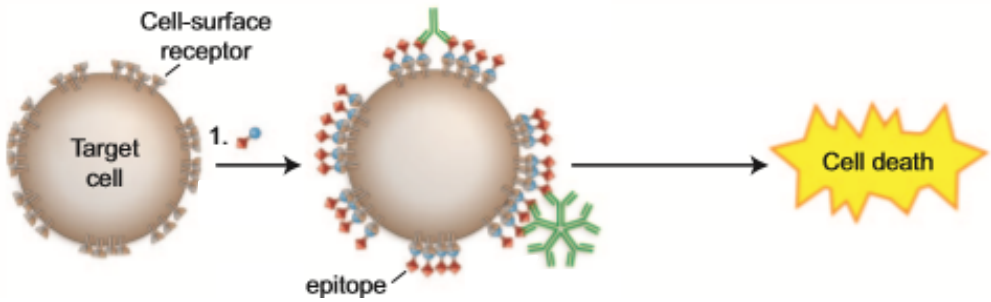

Figure 1: Process of small molecule recruiting antibodies to a diseased cell causing destruction by the immune system[2]

To create an effective small molecule to recruit endogenous antibodies, a sequence of peptides was synthesized using solid phase peptide synthesis with Fmoc chemistry and purified using HPLC. The first peptide created had four main elements: Two antibody specific targeting ligands with different specificities, a secondary antibody ligand that recognized any antibody, and a reactive functional group to covalently conjugate the peptide to the target antibody. This molecule was engineered to bivalently bind to one antibody, promoting covalent conjugation with the reactive functional group, and then monovalently bind to a second one with the second antibody specific ligand.

The first task in testing this molecule was to make sure that it covalently bound to the first antibody. Varying concentrations of the peptide were incubated with the antibody at a range of temperatures, and those that were left at 37˚C overnight achieved the greatest peptide to antibody binding, as high as 0.64 conjugations per antibody. By utilizing a shorter spacing between the specific targeting ligand and the secondary binding moiety, it is likely that this ratio would improve even more.[3] The peptide was also incubated separately with the same concentrations of other antibodies to test the selectivity of the covalent binding. As expected, greatly decreased to negligible binding was observed with the other antibody samples, suggesting that the peptide had a high selectivity for the targeted antibody.

In order to determine this binding ratio, the peptides were fluorescently tagged with FITC and incubated with their corresponding antibodies. After filtering out of the excess peptide, the absorbance spectrum of a sample was taken using spectrophotometry. Utilizing the absorbance peaks and the extinction coefficients for the FITC molecule on the peptide and for the antibodies, the concentrations of each were calculated. An ELISA was conducted to confirm the number of FITC's present.

Once the peptide was conjugated to the antibody, the next question was whether it could effectively link the two antibodies together. This was tested by measuring the effective diameter of a solution of both antibodies with the peptide using dynamic light scattering. A significant increase in effective diameter was observed compared with samples without the peptide.

After some observed success with binding two antibodies together, a new peptide was designed and synthesized to redirect an antibody to a second antibody on a cell. Due to the nature of this new targeting moiety, the synthesis of this peptide was designed to add the reactive functional group after the peptide was cleaved from the resin. Unfortunately, mass spectroscopy showed that attempts to add the functional group in this way resulted in an impure product, making it impossible to isolate a usable amount of the intended molecule. This could have resulted because of multiple undesired conjugations with the now deprotected side chains of the other amino acids in the peptide. A new synthesis method was therefore developed to be able to add this functional group before the peptide was cleaved from the resin. This resulted in the collection of the desired product, which was confirmed with mass spectroscopy.

For the next peptide, to target the antibody to a cell and test how well this binding causes the destruction of the cell, a new cell receptor, and thus a new targeting ligand was chosen. To be able to conjugate this ligand onto the peptide, a method was developed to form an ester bond with a hydroxyl group present on the ligand, resulting in a free carboxylic acid which could be reacted with the primary amine on the peptide.[4] The product was purified via HPLC and confirmed with mass spectroscopy.

Looking forward, future experiments will include flow cytometry to determine antibody conjugation to cell-surface receptors and also if there is uptake into the cells. It may also include assays to determine how effective future designs are at stimulating immune cell-mediated killing. These tests may lead to the development of a small molecule to securely redirect the body's own antibodies to fight disease.