My Research: Summer of 2010

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Part 1: My Story

I guess I was always meant to be a scientist. My aunt who used to babysit me could entertain me for hours with nothing but a glass of water, some spices, and a spoon. Performing my independent research, however, was the first time I ever did science for the purpose of helping others. My family does not have the best genes out there – we have a history of a variety of diseases that are so far untreatable. To illustrate, I just returned from my grandfather's funeral. He, like his son twenty years ago, succumbed to cancer. The feeling of helplessness as debilitating treatments did nothing to cure his disease bothered me from the time of his diagnosis in middle school to his eventual passing four days before my high school graduation.

I decided that cancer therapy, and by extension all medical treatments, could be done better. My interest in biology narrowed to an interest in medicine, and I started to look for ways to get involved. After my sophomore year, I was fortunate enough to volunteer at Hunter Laboratories, a diagnostic lab that analyzes blood and tissue samples from a broad area for regular checks and a host of diseases. I found this fascinating, but while my time there introduced me to the field, I was not satisfied by reacting to these illnesses. I'll never forget the day I watched my mentor run a young woman's sample over and over hoping he was drawing a false positive result for HIV. I realized that all we were doing to help was telling her she was sick. I wanted to do more.

The next summer, I looked for research positions in the hopes of developing new techniques and therapies. The Stanford Institutes of Medical Research, moderated by Dr. P.J. Utz and Cindy Limb, gave me the opportunity to perform an independent research project with a mentor. At the time I was choosing which of the five research areas in the program I wanted to join, my mother had begun experiencing arthritis symptoms, like her parents and grandparents already suffered from. Therefore, I chose immunology, which I had barely studied in the past. My mentor, Jordan Price, and I made friends right away, and he pushed me into further researching not only my work, but also others' in broader topics as well. I am so grateful to him and the other members of the Utz Lab who helped me perform my research and edit my report when the time came for submission. Their collective enthusiasm in welcoming a high school student into the lab and then helping him succeed is truly amazing to me.

Part 2: The Science

Introduction

About Lupus

Systemic Lupus Erythematosis (SLE or lupus) is a chronic autoimmune disease affecting 1.4 million Americans, 9 in 10 of whom are women according to Lupus.org. The Lupus Foundation estimates that women of color have two to three times the average likelihood of developing SLE. Lupus affects every patient differently, but 85 percent of patients survive over ten years with the disease. Still, however, the causes of the disease and its symptoms are unclear, and further research will be required before new treatments can develop. Some symptoms of lupus include inflammation, butterfly rashes, and a higher

risk of developing other diseases like arthritis, which are chemically similar. In serious cases, lupus patients experience clogged kidneys and need transplants.

Lupus appears to be caused by an overactive immune system that cannot distinguish which substances belong to the body and which are foreign and must be neutralized. Currently, the only way to treat lupus is to combat the inflammation with prednisone or shut down the immune system entirely with drugs called immunosuppresants. While these therapies are effective, they only treat the symptoms, rather than addressing the root causes of the disease.

How it works

Lupus is an autoimmune disease, meaning that the body's immune system attacks itself the way it would neutralize a foreign invader. The weapons of choice are antibodies, proteins that are shaped to recognize and attach to a certain substance, thereby labeling it to be destroyed by white blood cells. These antibodies can be shaped to match a variety of chemicals, and in lupus, these are often the remnants of cells that have died. One way this causes problems to a lupus patient is that when an antibody binds to a molecule, the large complex that is formed can clog up the kidneys and heart, sometimes requiring a transplant. This, however, is only one way that antibodies can hurt a lupus patient.

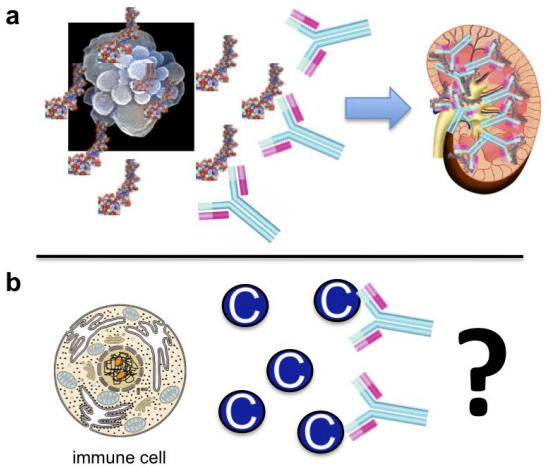

Figure 1: Autoantibodies in SLE. (**a**) Antibodies form immune complexes with materials left over from dead cells and get stuck in the kidneys and other organs. (**b**) Antibodies have also been shown to bind to cytokines and chemokines, the messenger molecules of the immune system.

In other autoimmune diseases similar to lupus, antibodies have a different target: the immune system itself. The chemicals targeted are cytokines and chemokines, the

small chemical messengers that the immune system's cells send to each other to communicate. Cytokines and chemokines play a critical role in coordinating the cells of the immune system to produce a unified response to invaders, and disrupting these pathways can have far-reaching implications for a patient.

Hypotheses and Theoretical Propositions

The resources I was lucky to have available to me allowed me to test multiple elements of the interaction between antibodies and communication molecules in lupus. The hypotheses I examined were as follows:

First, lupus patients produce more antibodies as their disease symptoms get worse. In other words, antibody levels are correlated with the overall severity of the disease. While this may not indicate that antibodies directly cause the disease to get worse, it does provide evidence of a connection that doctors can use to better understand their patients' progress.

Second, lupus causes the production of antibodies against cytokines and chemokines. I predicted that the antibodies to these messenger chemicals would be just as prevalent as those to the antigens already known about. This would provide a whole new category of chemicals for scientists to investigate in researching lupus. While certain cytokines and chemokines are known to have a role in lupus, antibodies against them are still being researched.

Thus, I hypothesized that profiling the blood of lupus patients would reveal previously undiscovered cytokine- and chemokine-targeted antibodies.

Materials and Methods

Blood Samples

Fortunately, a previous study in my lab had saved mouse samples from experiments done in 2004. The samples consisted of serum (blood with the cells and platelets removed) that had been drawn from the mice periodically over the course of their lives. Therefore, examining different time points of blood drawings would give an accurate picture of how the disease progressed in them over time. I had access to serum from three strains of mice: two that were genetically altered to have autoimmune diseases, and one that contracted lupus-like symptoms after being injected with a mineral oil called pristane.

Measurement

To figure out which antibodies the mice had in their blood, I used antigen arrays, a new technology developed in the Utz Lab at Stanford University. These take advantage of the binding properties of antibodies to identify their targets. As shown in figure 2, the arrays start off as microscope slides with pads of nitrocellulose – a weblike substance that non-specifically binds. In other words, it sticks to anything. Then, a machine prints spots of certain chemicals onto the pad, and they stick. This completes the production step of the array and prepares it to be processed.

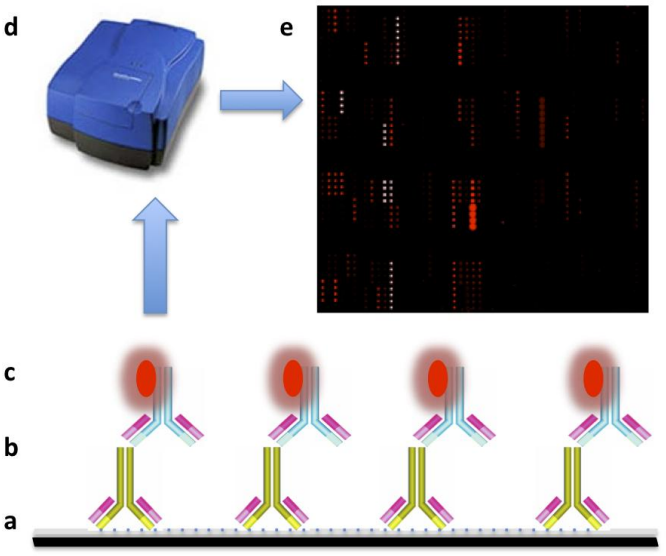

Figure 2: Array processing. (**a**) Chemicals were printed in spots onto sticky slides and non-printed areas of the slide blocked with non-fat milk. (**b**) Mouse serum was applied to the array, allowing antibodies shaped these features to bind. (**c**) A second antibody that recognizes any mouse antibody bound to the array was applied. This antibody was labeled with a florescent marker. (**d**) Laser scanning system produces digital images of florescent spots. (**e**) Images were analyzed to note which chemicals were attacked by antibodies based on the presence of florescent markers.

The first step in processing is to wash the array with milk, which sticks to the nitrocellulose so that all that is left sticking out is the printed spots of chemicals. The

next step is to incubate the arrays with the blood samples. Any antibodies that are targeted toward a chemical on the array will bind to the corresponding spot. Then the arrays are washed so that any other chemicals in the blood go away, leaving only the spots of antigen with antibodies bound to the top of them. The detection step is to add another antibody, called a secondary. It has a florescent molecule attached to it that gives off light under a laser. Instead of being targeted to a chemical we printed on the array, it binds to the antibodies in the blood. Then, after washing, all that is left are spots of chemicals that were never recognized and therefore blank, and spots that were recognized and now have two layers of antibodies on top of them.

The data step of running an array uses a laser instrument and a computer. The laser shines down on the array and takes a picture of the florescence. Keep in mind that any spots where the secondary antibody has bound will light up. A computer then interprets the picture and converts the image into a digital florescence intensity reading. By seeing which spots light up, we can accurately identify the chemicals that the blood serum had antibodies to.

Results

The results from each of the three mouse models confirmed my hypothesis. The arrays identified multiple cytokines and chemokines that mice produce antibodies against. One of these antibodies is called B Cell Activating Factor (BAFF), and is currently a target for treatments that have advanced to clinical trials. Among the other cytokines and chemokines, antibodies were produced more and more as the disease went on – at similar rates as the antibodies to known targets in lupus. Therefore, we can

conclude that antibodies against communication molecules in the immune system play an integral part in the chemical progression of lupus.

Future Steps

I just described the research project I performed last summer and submitted to the Intel Science Talent Search. This summer, I am pursuing my research in the same laboratory. One of the chemicals we identified as a lupus target was B-Cell Activating Factor (BAFF), which has been targeted by the most cutting-edge lupus drugs, but whose specific activities remain mysterious. Jordan and I are trying to characterize the biochemical pathways BAFF is involved in to see if it plays a role in initiating or perpetuating lupus. To do this, we are developing methods other than the microarrays from last summer that we can use to measure BAFF in a quantifiable way as well as in different contexts other than blood serum.

Next year, I will be studying Biological and Chemical Engineering at Princeton University, and hope to enter the pharmaceutical industry in the future. My dream is to found my own company and work to treat rare and little-studied diseases. Thanks for reading and good luck to you in your own science endeavors!