Peter Delaney

Strnad Final Report

Over the past three summers, I have committed a significant amount of my time to working as a student researcher in a lab at Case Western Reserve University. Before I began working at the lab after my freshman year of high school, I had no prior research involvement or training that helped me with this project. I had to read abstracts, papers, and other materials before beginning and I had to shadow and learn from post-docs in Dr. Pizarro's lab. Specifically, I learned how to isolate Peripheral Blood Mononuclear Cells (PBMC) from Inflammatory Bowel Disease (IBD) patient blood, harvest the Mesenteric Lymph Nodes (MLN) from mice, count and then stain cells, properly handle mice and hazardous materials, use the Flow Cytometry machine software and analyze and interpret those graphs. I taught myself how to make graphs using FlowJo software as well. Before being allowed handle animals, I also had to complete online training modules and tests and attend a facility tour and basic rodent handling training session. After being taught these specialized procedures, I was left alone to perform the experiments on my own.

After completing background research on Interleukin-33 (IL-33), Interleukin-17 (IL-17), the SAMP mouse model, and Crohn's Disease (CD), I developed my own protocol, which Dr. Pizarro and her post-doc, Rekha Garg, then modified. This allowed me identify the major IL-17-producing cell populations using isolation techniques and Flow Cytometry, and identify the effect of IL-33 administration on IBD patient blood cells and mice. I developed my own protocol to achieve my aims, which Dr. Pizarro and her post-doc, Rekha Garg, then adjusted and corrected.

The aim of my study was to investigate the potential effect of IL-33 and IL-17 in SAMP mice and patients with IBD, as well as identify which cells produced IL-17 when stimulated by IL-33.

I conducted four experiments to determine the conclusion. First, recombinant IL-33 was administered daily to 12-week-old SAMP mice for a one-week period. The mice were sacrificed and the MLN's were harvested and analyzed for differences between IL-33 treated and control mice. In unfractionated MLN's from IL-33-treated SAMP, we found an increased percentage of CD11b+F4/80+ cells, representing an activated macrophage population, compared to vehicle treated SAMP. In addition, a significant increase in the production of the Th17 cytokines, namely, IL-17A, as well as IL-22, was observed. Taken together, these data suggest that early IL-33 dependent events in an animal model susceptible to colitis may consist of expansion of an activated macrophage cell population and the production of macrophage-associated proinflammatory and Th17-type cytokines.

The next experiment that I conducted attempted to block intestinal inflammation to see if inflammation could be reversed. We utilized the strategy of neutralizing IL-33 function by administering an anti-ST2 antibody that blocks IL-33 signaling through the cell-associated receptor, ST2L. We administered the antibody to 12 week old SAMP mice twice weekly over a six-week period, after which the mice were sacrificed at 18 weeks of age. The treatment protocol significantly decreased the total inflammation in anti-ST2 treated SAMP, and showed an overall improvement in inflammation and restoration of the overall villous architecture of the ileum of the mice. We also analyzed the mice for macrophage populations and found that the *in vivo* IL-33 neutralization that was conducted reduced the overall percentage of IL-17A-producing CD 11b+F4/80+ cells (macrophages) within the MLN's.

The next experiment supported these findings, wherein IL-33 had the ability to increase percentages of IL-17A-producing CD11b+F4/80+ cells from activated MLN's in both SAMP and control AKR mice, but more potently in the SAMP mice.

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The final experiment was performed *in vitro* with PBMC's from active Crohn's Disease (CD) patients. We observed similar trends found in SAMP mice wherein IL-33 had no effect on IL-17 production in normal, healthy controls. However, a significant increase in the percentage of IL-17-producing CD11b+CD68+ cells was seen after IL-33 stimulation specifically among CD patients.

In summary, my project showed that *in vivo* administration of IL-33 to SAMP mice enhances innate immunity and expands activated macrophages. Second, that IL-33 increases IL-17A production from unfractionated MLN cells from SAMP mice and PBMC from CD patients. Third, that the blockade of the IL-33/ST2 axis ameliorates intestinal inflammation in inflamed SAMP mice, and finally, that IL-33 neutralization specifically reduces the IL-17A expressing activated macrophage population. In conclusion, the IL-33/ST2 axis represents a novel, signaling pathway that may play an important role in the pathogenesis of intestinal inflammation, and IL-33 appears to selectively expand a potentially pathogenic cell population consisting of IL-17A-producing macrophages.

I wanted to investigate the potential effect the cytokine (signaling-protein) IL-33 had on the production of IL-17 in a mouse model and in patients with inflammatory bowel disease (such as Crohns disease) and to identify which cells were producing IL-17 when stimulated by IL-33. I administered IL-33 to the blood cells of IBD patients and mesenteric lymph node cells from mice after having activated the cells. I also injected recombinant IL-33 into mice and then sacked them to observe the inflammation. Finally, I injected a ST2-Fc protein Peripheral blood mononuclear cells (PBMCs) from CD patients with active disease and from healthy controls (Ctrls), and MLN from SAMP and (parental) control AKR mice were activated with anti-CD3/anti-CD28 and cultured in the presence/absence of IL-33 (20 ng/ml) and evaluated by FACS. rmIL-33 was administered daily (one wk) and ilea from experimental mice were histologically-assessed for inflammation; MLN were evaluated by FACS or cultured/analyzed after anti-CD3/anti-CD28 activation for cytokine profiles. IL-33 blockade was achieved by twice-weekly administration (4 wks) of an ST2-Fc protein. IL-33 selectively expands a potentially pathogenic cell population consisting of IL-17A producing macrophages. The IL-33/ST2 axis represents a novel signaling pathway that may play an important rôle in the pathogenesis of intestinal inflammation

I have found it incredibly fulfilling to work on a project that has real-world applications. It has been even more meaningful that my discovery might provide new avenues to fight a terrible disease with the hope of improving the lives of the 1.4 million Americans that suffer from the disease.

I learned how to isolate PBMC's from IBD patient blood, harvest the MLN from mice, count and then stain cells, properly handle mice and hazardous materials, use the Flow Cytometry machine software and analyze and interpret those graphs. I taught myself how to make graphs using the FlowJo software. After being taught these specialized procedures, I was left alone to do it all by myself.
After doing background research on IL-33, IL-17, the SAMP mouse model, and Crohn's disease, I developed my own protocol, which Dr. Pizarro and her post-doc, Rekha Garg, then modified. This allowed me identify the major IL-17 producing cell populations using isolation techniques and Flow Cytometry, and identify the effect of IL-33 administration on IBD patient blood cells and mice.

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I designed a project that investigated the effect of an immune system signaling-protein called IL-33 on the development of IBD. I used a special genetic mouse model of the condition (the SAMP mouse) and blood cells from patients with IBD. I stimulated the diseased cells with IL33 and proved that it triggered the disease through another protein called IL17, by activating a cell called a macrophage, which was also previously unknown. My findings demonstrated a new signaling pathway for IBD and suggest that the IL33/IL17 interaction may be a new target for treating Crohn's disease and ulcerative colitis.

In the USA alone, there are 4 million people with inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis. I studied the effect of an immune system signaling-protein called IL-33 on the development of IBD. I used a special genetic mouse model of the condition (the SAMP mouse), and blood cells from patients with IBD. I stimulated the diseased cells with IL33 and proved that it triggered the disease through another protein called IL17, by activating a cell called a macrophage. I performed other tests to isolate and confirm the mechanisms involved. My findings demonstrated a new signaling pathway for IBD, and suggest that the IL33/IL17 interaction may be a new target for treating Crohn's disease and ulcerative colitis.