ABSTRACT

Elizabeth Goodall Rossitch
The Effect of COPD on the Immune Response to Influenza Virus Vaccination
(Under Direction of Melinda A. Beck)

Chronic Obstructive Pulmonary Disease (COPD) is a health concern worldwide and particularly in the United States. It is the currently the fourth leading cause of death in the world and is predicted to become the third leading cause of death by 2030. People with COPD often experience exacerbations, an acute onset of more severe symptoms, caused by infections-bacterial and viral. These exacerbations decrease quality of life and further disease progression. Not to mention, while healthy individuals can easily recover from an influenza, it could be deadly in COPD patients. Thus, it is important to ensure that individuals with COPD are adequately covered by influenza vaccination. Currently, little is known about how COPD impacts the cell-mediated immune response, but gaining this knowledge would help determine if individuals with COPD are effectively covered by the vaccine.

The purpose of this pilot study was to determine if COPD affects the cell-mediated immune response post influenza vaccination. Specifically, it aimed to discover if the percentage of CD3+CD8+ T cells were the same in individuals with and without COPD. Additionally, it aimed to assess the ability of these CD3+CD8+ T cells to secrete IFNγ and TNFα. A total of 8 participants were included in the analyses of this study- 3 with COPD and 5 with no respiratory history. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples 28-35 days post-vaccination and incubated for 120 hours with and without (in two separate wells) the
A/Victoria/361/2011. The cells were stained for CD3, CD8, TNFα, and IFNγ using human fluorochrome-conjugated human antibodies.

There was no significant difference in the CD3+CD8+ T cell percentage between COPD and non-COPD groups, but the percent increase of CD3+CD8+ T cells secreting TNFα and IFNγ when stimulated with *in vivo* with A/Victoria/361/2011 was significantly greater in the non-COPD group. This suggests that there might be a problem in CD3+CD8+ T cell function in individuals with COPD. Therefore, there need to be further studies to determine whether COPD patients are adequately covered by the current influenza vaccination.
ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Melinda A. Beck, because this project would not be possible without her and Dr. Patti Sheridan for her continued interest and support in my project. I would also like to thank my graduate student mentors Jennifer Rebeles, Justin Milner, Heather Paich, and Scott Neidich for their patience as they shared their skills and knowledge with me and helped every step of the way. Finally, I would like to thank all of the Beck lab for your support during the completion of this project.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>APC</td>
<td>allophycocyanin</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic Obstructive Pulmonary Disease</td>
</tr>
<tr>
<td>CTL</td>
<td>cytotoxic T Cell</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hank's Balanced Salt Solution</td>
</tr>
<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cells</td>
</tr>
<tr>
<td>PE</td>
<td>phycoerythrin</td>
</tr>
<tr>
<td>PerCP</td>
<td>peridinin-chlorophyll-protein complex</td>
</tr>
<tr>
<td>TIV</td>
<td>trivalent inactivated influenza vaccine</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Chronic Obstructive Pulmonary Disease (COPD)

Chronic Obstructive Pulmonary Disease (COPD) is predicted to be a worldwide health concern over the next few decades. Currently it is the fourth leading cause of death, but the World Health Organization projects that COPD will move to third by 2030 (3). A literature review extracting prevalence data from 80 articles found that the United States had the highest COPD prevalence of 37%, compared to Japan which had the lowest prevalence of 0.2% (4).

COPD is a life threatening lung disease that disrupts normal breathing through long-term cough with mucus build up (chronic bronchitis) or destruction of the lung tissue over time (emphysema). Symptoms of COPD develop slowly over time and include dyspnea, chronic cough, abnormal sputum, and trouble doing normal daily activities such as walking up a flight of stairs. The number one risk factor for COPD is smoking tobacco, but other risks include certain gas or fume exposures in the workplace, secondhand smoke or pollution exposure, and exposure to cooking fire without proper ventilation (5).

COPD cannot be cured, but symptoms can be treated via medication. COPD patients can take short-acting beta-2 agonists such as Albuterol for acute symptoms and long-acting beta-2 agonists such as Salmeterol for long term management of symptoms. These medications cause bronchodilation which relieves symptoms and makes breathing easier. In addition, disease progression can be slowed by decreasing
exacerbations which aggravate the lungs, increase death in COPD patients, and decrease quality of life. An exacerbation is a period (at least 48 hours) where relatively more severe symptoms are experienced by the patient. So what causes these exacerbations? There are several suggested causes such as non-adherence to medication, heart failure, pneumonia, inhalation of irritants such as tobacco smoke, and most notably bacterial and viral infections (3).

1.2 Influenza Infection

The flu season typically lasts from October to March and The Centers for Disease Control and Prevention (CDC) estimates that during this time 5 to 20 percent of people in the United States are infected with influenza (6). This may not seem like an alarming statistic because many people can recover from flu without medication or complications (7). Despite this, the seasonal influenza hospitalizes more than 200,000 and kills more than 36,000 Americans each year (6). People at greater risk for hospitalization and death from influenza infection include children younger than 5 years old, older adults greater than 65 years old, pregnant women, and people with chronic illnesses such as COPD (7).

Influenza, or flu, is a contagious viral infection, characterized by a sudden rise in body temperature (greater than 38.5°C), headache, muscle aches, fatigue, and cough (8). It is transmitted person to person directly or indirectly. Direct transmission occurs when an infected individual coughs, sneezes, or talks, releasing tiny virus-containing droplets into the air that are inhaled by the recipient via nose or mouth (6). These droplets then enter the nasal and laryngeal mucosae where the virus can infect nasal cells, replicate, and continue spreading down into the lower airway (8). Indirect
transmission occurs when the recipient touches virus from these droplets that remains on a surface or object and then subsequently touches their mouth, eyes or nose. Viruses can last on surfaces between two and eight hours depending on conditions (6).

1.3 Cellular Immune Response to Influenza and Importance of Vaccination

The body has several lines of defense to protect against infection, including mucus production to prevent it in the first place, however this paper will focus on the cell mediated response specifically for influenza. When a specific strain of influenza infects for the first time, the body depends on the innate immune system to respond. Later in the infection, the adaptive immune response responds, and unlike innate immunity, the adaptive immune response contains memory cells that can quickly respond to reinfection with the same viral strain. The goal of vaccination is to induce the production of memory T cells and B cells, necessary for a rapid adaptive immune response. Upon creation of these cells, the body is able to respond more quickly and effectively if the same pathogen infects again. This is called a secondary immune response (9).

Cellular immunity against influenza viral infections relies on dendritic cells, which are known as the antigen-presenting cells and can be found surrounding the epithelial cells of the airway. When dendritic cells recognize and ingest influenza infected cells, they migrate to the lymphatic system where they present influenza virus-derived antigens to the T cells, activating them. Specifically, CD8+ cytotoxic T cells (CTLs) recognize viral proteins presented in a complex with major histocompatibility complex (MHC) class I molecules in the dendritic cell membrane while CD4+ helper cells recognize viral proteins associated with MHC class II molecules in the cell membrane. CTLs then migrate to the site of infection where they recognize and induce apoptosis in infected
cells. They also indirectly inhibit viral replication by secreting cytokines such as interferon-γ (IFNγ) and tumor necrosis factor-α (TNF-α) (10).

1.4 COPD and Influenza Virus

If the number of COPD exacerbations were decreased, mortality rates would decline and quality of life would improve among COPD patients. Since 78% of exacerbations in all patients admitted to the hospital were caused by a viral or bacterial infection, it is imperative that we aim to prevent these infections, especially in COPD patients (3). A virus many of us may consider to be non-fatal could be potentially deadly in COPD patients. This paper aims to determine if there are any changes in CD8+ cytotoxic T cell function that might make patients with COPD more susceptible to flu.
CHAPTER 2

SPECIFIC AIMS AND HYPOTHESIS

**Specific Aim:** To determine if Chronic Obstructive Pulmonary Disease affects cell-mediated immune response post influenza vaccination

**Hypothesis:** COPD will result in an impaired cell-mediated immune response. Specifically, I hypothesize that, following *in vitro* stimulation with influenza virus, fewer CD8+ T cells will secrete IFNγ and TNFα. I hypothesize that the percentage of CD8+ T cells will be lower for individuals with COPD than individuals without COPD.
3.1 Study Population and Samples:

This study population was chosen from individuals participating in a prospective observation study being carried out at the University of North Carolina at Chapel Hill Family Medicine Center. Specifically, we targeted subjects who were followed during the 2012-2013 flu season. Adults 18 years or older who were scheduled to receive the 2012-2013 trivalent influenza vaccine (TIV) at the UNC Chapel Hill Family Medicine Center were eligible for participation. Patients were excluded based on the following criteria: immunosuppression, self-reported use of immunomodulator or immunosuppressive drugs, acute febrile illness, history of hypersensitivity to any influenza vaccine components, history of Guillain-Barre syndrome, or use of theophylline preparations or warfarin (1).

After receiving the 2012-2013 TIV in the deltoid muscle, the subject was asked to participate in the study. Informed consent was obtained and the study nurse measured each participants’ height and weight and obtained a blood sample. The FDA approved the following influenza strains for inclusion in the 2012-2013 influenza vaccine: A/California/7/2009 (H1N1)-like virus, A/Victoria/361/2011 (H3N2)-like virus, B/Wisconsin/1/2010-like virus (2). Twenty-eight to thirty-five days after receiving the 2012-2013 influenza vaccination, participants returned to the clinic to have another blood sample taken. We isolated PBMCs from the post-vaccination blood samples referred to here.
From this larger study, 25 individuals were selected for inclusion into this pilot study. Fifteen of the samples (6 COPD and 9 non-COPD) were lost due to a staining failure on this particular plate. Of the remaining 10 samples, 1 COPD sample and 1 non-COPD sample were lost due to lack of cells remaining at the end of the staining process. Thus, of the 8 participants that remained, 3 had a diagnosis of COPD and 5 had no COPD diagnosis. The medical records of patients classified as having no COPD diagnosis were analyzed to ensure that there was no history of any respiratory difficulties. Diabetes was defined as either having a current diagnosis of diabetes or having no diabetes diagnosis. Each participant was defined as either a current smoker, past smoker, or never a smoker. Smoking status was self-reported by the patients.

**3.2 Peripheral Blood Mononuclear Cell Collection:**

Post-vaccine blood samples were stored at 4°C and then moved to room temperature 30 minutes prior to the PBMC isolation process. Histopaque-1077 was brought to room temperature and 3mL were aliquoted into a 15mL conical centrifuge tube. Up to 8mL of whole blood was layered on top of the Histopaque-1077 and the conical tube was centrifuged at 400 X g for 30 minutes at room temperature. The following distinct layers were identified starting from the bottom to the top: red blood cells, Histopaque-1077, PBMCs, and plasma. A pipet was used to extract the PBMC layer and transfer the PBMCs into a new 15mL conical centrifuge tube. The cells were suspended in 10 mL sterile Hank’s Balanced Salt Solution (HBSS) and centrifuged at 250 X g for 10 minutes at room temperature. The supernatant was discarded and the cells were suspended in 5mL HBSS before being centrifuged at 250 X g for 10 minutes.
at room temperature. The cells were suspended in HBSS one final time (3 total washes in HBSS).

The supernatant was discarded and the cells were suspended in 500µL of Serum Albumin Solution that was kept at 4°C until use. This solution was transferred to a 2mL cryogenic vial and 500µL of Freezing Medium was added dropwise. The vials were placed inside isopropanol-filled Mr. Frosty containers and stored at -80°C for at least four hours. The cells were then moved in their vials to the liquid nitrogen tank until thawed for specific studies.

3.3 PBMC Thawing, Plating, and Stimulation:

The PBMCs from my 8 samples and a control sample (the control had received the 2012-2013 TIV, was 25 years old, and had a healthy weight BMI) were thawed, counted, and diluted with AIM-V media. The cells were diluted to a 1X10^6 cells/200µL media solution. From each sample, 1X10^6 cells were plated into two separate wells on 96-well round-bottom plate by pipetting 200µL of solution to each of the two wells (one to be stimulated and one to be left unstimulated). This held true for all the samples with the exception of 04-1012 and 04-1488 because there were fewer than 2X10^6 cells. For each of these samples 1X10^6 cells were plated in the stimulated wells and 5.6X10^5 and 6.87X10^5 cells were plated into the unstimulated wells for samples 04-1012 and 04-1488 respectively. The cells were rested for 2 hours in the incubator at 37°C and 5% CO₂.

The plate was centrifuged at 350 X g for 5 minutes and the media was pipetted from the wells. Stimulation wells received 20µL of a 1:3 dilution of live
A/Victoria/361/2011 (H3N2) virus and 80µL AIM-V media (a total dilution of 1:15), while control wells received 100µL AIM-V media. Cell were incubated at 37°C and 5% CO₂ for 114 hours. Cells were centrifuged and stimulated wells received 79µL AIM-V media, 1µL GolgiPlug, and 20µL of the 1:3 dilution of live H3N2 virus. Unstimulated wells received 99µL AIM-V media and 1µL GolgiPlug. Cells were then incubated for 6 more hours at 37°C and 5% CO₂.

3.4 PBMC Staining and Flow Cytometry:

PBMCs were stained extracellularly for CD3 and CD8 and intracellularly for IFNγ and TNFα. Extracellular staining was performed by spinning the cells down, removing the media and adding 100µL of extracellular master mix presented in Table 1 below. The plate was covered and incubated at 4°C for 30 minutes before adding 100µL of FACS buffer to each well and mixing. The cells were centrifuged at 350 x g for 5 minutes at 4°C and supernatant was flicked gently into the sink. These steps were repeated with 200µL of FACS buffer and the cells were scraped off the bottom of each well.

**Table 1: Extracellular staining mix recipe per one sample**

<table>
<thead>
<tr>
<th>CD3 PerCP-cy5.5 (µL)</th>
<th>CD8 FITC (µL)</th>
<th>FACS Buffer/FcR block mix (µL)</th>
<th>Total Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5</td>
<td>90</td>
<td>100</td>
</tr>
</tbody>
</table>

* The FACS Buffer/ FcR block was a 1:100 dilution of FcR block

The PBMCs were then fixed and permeabilized before intracellular staining. To do this 100µL of Cytofix/Cytoperm was added and mixed in each well. The plate was covered in foil and incubated at 4°C for 20 minutes. The plate was removed from the refrigerator and 100µL of Perm/Wash was added and mixed in each well. The cells
were centrifuged at 350 x g for 5 minutes at 4°C and supernatant was flicked gently into the sink. The cells were washed again with 200µL Perm/Wash, centrifuged at the previous settings, and supernatant was removed.

Next the cells were stained intracellularly by adding 100µL of the master mix presented below in Table 2. The plate was covered in foil and incubated at 4°C for 30 minutes before. Next 100µL of Perm/Wash was added and mixed in each well before covering the plate in foil and centrifuging it for 5 minutes at 350 x g and 4°C. Supernatant was removed. Next 200µL of FACS buffer was added and mixed in each well, gently scraping the cells from the bottom of the well. The plate was covered in foil and centrifuged at 4°C, 350 x g, for 5 minutes. Supernatant was gently flicked off and 100µL of FACS buffer was added to each well (scraping cells gently while mixing). This FACS buffer was transferred to a dilution box and 100µL more of FACS buffer was added to each well so that any remaining cells could be scraped from the bottom of each well. The FACS buffer was transferred to the same dilution box. Each sample was vortexed before being analyzed using a BD Accuri Flow Cytometer and the data was analyzed using FlowJo.

### Table 2: Intracellular master mix recipe per one sample

<table>
<thead>
<tr>
<th>TNF-α PE (µL)</th>
<th>IFNγ APC (µL)</th>
<th>Perm wash (µL)</th>
<th>Total Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5</td>
<td>90</td>
<td>100</td>
</tr>
</tbody>
</table>

### 3.5 Statistical Analysis:

The flow cytometry data was analyzed using JMP statistical software. All of the analyses were performed using a two-tailed Kruskal-Wallis test. A p-value of less than 0.05 was considered to be significant.
CHAPTER 4

RESULTS

4.1 Demographics of the Study Population:

Participants were classified into two groups: COPD and no COPD. The COPD group is characterized by individuals with a diagnosis of COPD. The no COPD group is characterized by individuals who had no respiratory medical history. The demographics of each participant are shown in Table 3 below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sex</th>
<th>Race</th>
<th>Age</th>
<th>BMI</th>
<th>Diabetes</th>
<th>Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04-1220</td>
<td>Female</td>
<td>White</td>
<td>56</td>
<td>32.2</td>
<td>Yes</td>
<td>Past</td>
</tr>
<tr>
<td>04-1348</td>
<td>Male</td>
<td>White</td>
<td>58</td>
<td>27.3</td>
<td>Yes</td>
<td>Current</td>
</tr>
<tr>
<td>04-1415</td>
<td>Male</td>
<td>White</td>
<td>72</td>
<td>31.2</td>
<td>Yes</td>
<td>Current</td>
</tr>
<tr>
<td>No COPD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>04-1012</td>
<td>Male</td>
<td>White</td>
<td>65</td>
<td>23.1</td>
<td>Yes</td>
<td>Current</td>
</tr>
<tr>
<td>04-1342</td>
<td>Female</td>
<td>White</td>
<td>66</td>
<td>19.7</td>
<td>No</td>
<td>Current</td>
</tr>
<tr>
<td>04-1371</td>
<td>Female</td>
<td>White</td>
<td>60</td>
<td>29</td>
<td>Yes</td>
<td>Past</td>
</tr>
<tr>
<td>04-1399</td>
<td>Female</td>
<td>White</td>
<td>62</td>
<td>27.2</td>
<td>No</td>
<td>Never</td>
</tr>
<tr>
<td>04-1488</td>
<td>Male</td>
<td>White</td>
<td>76</td>
<td>25.1</td>
<td>No</td>
<td>Past</td>
</tr>
</tbody>
</table>

The average age in the COPD group was 62 ± 8.72, compared to the average age of 65.8 ± 6.18 in the group with no COPD. The average BMIs for the COPD group and No COPD group were 30.23 ± 2.59 and 24.82 ± 3.62 respectively.

4.2 CD3+CD8+ T Cells

There are no significant differences in the percentage of CD3+CD8+ T cells between people with COPD and people without COPD for either unstimulated or stimulated PMBCs (Figure 1).
There are no significant differences in the percentage of CD3+CD8+ T cells between people with COPD and people without COPD for either unstimulated or stimulated PMBCs (Figure 2). Yet, individuals without COPD trended towards a greater expression of CD3+CD8+IFNγ+ T cells when comparing the stimulated samples between the two groups. With the exception of one COPD participant, all individuals without COPD had greater CD3+CD8+IFNγ+ T cell expression than individuals with COPD after stimulation (Figure 3). Despite there being no statistical differences between CD3+CD8+IFNγ+ T cells in people with or without COPD, we did find that the
percent increase between unstimulated and stimulated PBMCs from individuals with COPD was significantly lower for CD3+CD8+IFNγ+ T cells (Figure 4).

Figure 2. There are no significant differences in the percentage of CD3+CD8+IFNγ T cells between people with COPD and people without COPD for either unstimulated or stimulated PMBCs.

Figure 3. All participants trended towards an increase in CD3+CD8+IFNγ+ T cell expression upon stimulation with H3N2, but individuals without COPD trended towards a greater expression CD3+CD8+IFNγ+ T cells after stimulation with H3N2.
**Figure 4.** Impaired upregulation of CD3+CD8+IFNγ+ T cells in PBMCs from individuals with COPD.

### 4.4 CD3+CD8+TNFα+ T Cells

Individuals with COPD had a significantly higher expression of CD3+CD8+TNFα+ T cells than individuals without COPD for unstimulated PMBCs. There was no difference between CD3+CD8+TNFα+ T cell expression in people with COPD compared to people without COPD for PBMCs stimulated with H3N2 (Figure 5). Despite this, we did find that the percent increase between unstimulated and stimulated PBMCs from individuals with COPD was significantly lower for CD3+CD8+TNFα+ T cells (Figure 6). All participants without COPD were able to upregulate their expression of CD3+CD8+TNFα+ T cells when stimulated with H3N2, while individuals with COPD varied in their response upon stimulation. Figure 7 shows the three different responses among COPD patients. One COPD participant upregulated expression of CD3+CD8+TNFα+ T cells, while the other COPD participants either had downregulated expression or unchanged expression of CD3+CD8+TNFα+ T cells upon stimulation.
Figure 5. Individuals with COPD had a significantly higher expression of CD3+CD8+TNFα+ T Cells than individuals without COPD for unstimulated PMBCs.

Figure 6. Impaired upregulation of CD3+CD8+TNFα+ T cells in PBMCs from individuals with COPD.
Figure 7. All individuals without COPD trended towards an increase in CD3+CD8+TNFα+ expression upon stimulation with H3N2, but individuals with COPD had different responses to stimulation.
CHAPTER 5
DISCUSSION

5.1 CD3+CD8+ T Cells

CD3+CD8+ T cell expression was similar in people with COPD and people without COPD for unstimulated PBMCs and PBMCs stimulated with H3N2. This suggests that the dendritic cells are recognizing influenza virus, migrating to the lymphatic system, and presenting influenza virus-derived antigens to T cells properly. Secondly, this indicates that CD3+CD8+ T cells are being activated appropriately, but there might be a problem in their function that causes people with COPD to be more susceptible to severe complications or even death when exposed to influenza.

5.2 CD3+CD8+IFNγ+ T Cells

PBMCs from each participant had an increased expression of CD3+CD8+IFNγ+ T cells when stimulated. In other words, PMBCs stimulated with H3N2 had more CD3+CD8+ T cells that were able to secrete IFNγ in response to influenza infection. This is important because IFNγ plays a role in inhibiting viral replication (13). But it is important to note that individuals without COPD had a significantly greater percent increase in CD3+CD8+IFNγ+ T cell expression upon stimulating PBMCs with H3N2. This indicates that there might be an impairment in the function of CD3+CD8+ T cells in people with COPD. Specifically, they could have impairments in the signaling pathway for the secretion of IFNγ in CD3+CD8+ T cells or there might be a defect in the IFNγ secretion pathway itself.

5.3 CD3+CD8+TNFα+ T Cells
Individuals with COPD had a significantly higher expression of CD3+CD8+TNFα+ T cells than individuals without COPD in unstimulated PMBCs. This was an expected finding because individuals with COPD have chronic systemic inflammation and TNFα is an important pro-inflammatory cytokine (11). Despite this, individuals with COPD and individuals without COPD had a similar percentage of CD3+CD8+ T cells with the ability to secrete TNFα when stimulated. So even though we found that individuals without COPD had a significantly greater percent increase of expression for CD3+CD8+TNFα+ T cells between unstimulated and stimulated PBMCs, the ability of CD3+CD8+ T cells to secrete TNFα may not be impaired in people with COPD. Instead, maybe people with COPD have decreased sensitivity to the anti-viral effects of TNFα and thus TNFα is not able to contribute significantly to the inhibition of viral replication (12). Or, there is a possibility that TNFα may not play a significant enough role in inhibiting viral replication and all of the TNFα pathways are normal in people with COPD.

5.4 Limitations

This study had several limitations. One of the greatest limitations of the study was the sample size. There were 8 total participants, 3 participants with COPD and 5 participants without COPD. Thus, the groups are too small to draw true significant differences between. Though we did find some significant differences, these among other trends observed can only point towards future research studies because of the small sample size.

An additional study limitation is that COPD status was determined based on medical records. Even though we ensured that each participant had a diagnosis of
COPD, we could not assess the severity or chronicity of each individuals’ COPD. People who were diagnosed with COPD more recently might not have the same immune response to influenza as people who have been diagnosed for a longer period of time. In other words, it may take time for some changes in the immune response to occur. In addition, people without COPD were chosen based on their medical records indicating no history of respiratory difficulties. Despite this, we cannot be sure that this person has not been seen for respiratory difficulties somewhere other than the Family Medicine Center or is just not reporting their symptoms to the physician.

Finally, the BMIs between the group with COPD and group without COPD are also a limitation to this study. People with COPD had an average BMI of 30.23 ± 2.59 and people without COPD had an average BMI of 24.82 ± 3.62. According to the National Institutes of Health (NIH), a healthy weight BMI is 18.5-24.9, overweight is 25.0-29.9, and obese is 30.0 and above (14). This means the No COPD group is considered to have a healthy weight BMI while the COPD group is considered obese. This is problematic because previous studies show that activation and function of CD8+ T cells are impaired in PBMCs from overweight and obese individuals exposed to influenza and there is impaired upregulation of TNFα secretion in PBMC supernates from obese individuals (1). So ultimately, the trends that we see here could be due to obesity instead of COPD.

5.6 Study Implications and Future Studies

People with COPD are more susceptible to severe complications or even death resulting from an influenza infection (3). Thus, we must ensure that COPD patients are adequately covered by the current dose of the influenza vaccine. To ensure adequate
coverage, future studies should look at CD3+CD8+ T cells activation and function just as we did here to confirm the trends presented in this paper. If in fact there are no significant differences in the percentage of CD3+CD8+ T cells between people with COPD and people without COPD for either unstimulated or stimulated PMBCs, it would be interesting to perform a study where a lung biopsy was performed in people who were actually infected with influenza. Differences in the expression of CD3+CD8+ T cells in the lung would indicate whether there is a problem in recruiting these cells to the lung during infection.

It would also be beneficial to perform studies that looked at how smoking status effects the cellular immune response to influenza virus vaccination because smoking is the number one risk factor for COPD (3). Because this study was not able to stratify the data based on smoking status, smoking could be a confounding factor in this pilot study. These studies would have huge clinical implications because if past-smokers had a similar cellular response to influenza stimulation as non-smokers or at least a better response than current smokers, it would give people yet another motivation to quit smoking.
References:


