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Christiana Daas
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THE EFFECTS OF E-VAPOR ON THE PATHOGENESIS OF RHEUMATOID ARTHRITIS
MOUSE MODELS AND POTENTIAL IMPLICATIONS FOR FUTURE GENERATIONS

by

Christiana Daas

Submitted to the School of Honors Committee
in partial fulfillment
of the requirements for University Honors Scholars

Southeastern University

2020
DEDICATION

The more I go through life the more I realize how uncommon it is to be so abundantly loved by two parents who love the Lord, love each other, and desire the best for their children. Therefore, I would like to dedicate this thesis to my parents, Mom and Papa. You two are more than I could ever have dreamed up on my own for supportive parents. Through every joy, every high, every low, every uncertain moment, every success and every bump in the road, you two have encouraged and supported my dreams. Thank you for seeking the Lord on my behalf and asking for favor in every area of my life. Thank you for your patience, for the endless fast food runs, and listening ears when I just needed to vent, go for a nature walk, or go shopping. You guys are the best.

Love, still your little girl
ACKNOWLEDGEMENTS

First and foremost, I must give credit where credit is due for the completion of this thesis. Without the Lord and his countless blessings, endless wisdom and gracious rest, I would have never been able to finish my thesis. After changing my thesis topic not once, but twice, I found myself sitting in front of my computer this July with zero pages of my thesis completed and the overwhelming feeling that this was an impossible task. However, nothing is impossible when you partner with the Lord, and prioritize your time around His agenda.

It also helps to have world-class advisors to help you through this process though. Dr. McConchie, thank you for your irreplicable insight concerning the field of immunology, and for partnering with me in this process on such short notice.

And you, Dr. Aimee Memaw Franklin PhD. You have changed my life. I have never met another human being as generous, patient, passionate, and sarcastic as you. “Thank you” falls ridiculously short of how grateful I am that the Lord brought you into my life. I know there are many days where it is easy to feel like an imposter, but the undeniable truth of the matter is that the Lord has used you to change countless students’ lives, the dynamic of the science department, and SEU for the better. I have seen these changes with my own eyes throughout my time being your “head” TA. You are an irreplicable person in my life. And since I’m graduated, I can finally say it. AIMEE Franklin, you are a dear friend and trusted mentor, thank you for being there during my joys, my struggles, and on the happiest day of my life. You have changed my life for the better, forever.

Since this acknowledgements page is officially a novel, I’d also like to thank my husband, Josh, from the bottom of my heart. You are the best husband ever- and though that is completely bias it is 100% correct. You are who my heart hoped for, and now I get to live every day of this amazing life by your side. Thank you for being steadfast and near me throughout every exam, every late-night study session, every breakdown and every accomplishment. I am so thrilled to see how the Lord uses us as we grow together in what He is calling us to. I love you so much. – Your best friend.
Abstract

Rheumatoid arthritis is the most prevalent autoimmune disease. Rheumatoid arthritis (RA) has no cure, and the direct cause of the disease is still unknown. The two leading hypotheses concerning its etiology are based on the effects of HLA-DRB1 gene expression, and cigarette smoke. Conjunctively, the use of vaping devices amongst adolescents has increased significantly since introduced in 2007. There is no long-term data on the effects of e-vapor and its aerosols on bodily health. Cigarette smoke is the most noteworthy environmental factor contributing to RA therefore the question is raised as to whether or not vaping relates to rheumatoid arthritis susceptibility. This extended literature review focused on the current knowledge of RA, as well as cigarette smoke and its role in rheumatoid arthritis pathogenicity. An experimental proposal is also described which analyzes the effects of e-vapor on collagen induced arthritis mouse models. HLA-DRB1 gene expression, autoantibody proliferation and rheumatoid arthritis symptomology were investigated in acute and chronic RA mouse models. Ideally, these experiments would clarify the potential effects of vaping in regard to RA and provide significant insight for future generations.

Keywords: Rheumatoid arthritis, environmental factors, vapor, cigarette smoke, HLA-DRB1
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ACPA</td>
<td>Anti-citrullinated Protein Antibody</td>
</tr>
<tr>
<td>APC</td>
<td>Antigen Presenting Cell</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
</tr>
<tr>
<td>CFA</td>
<td>Complete Freunds Adjuvant</td>
</tr>
<tr>
<td>CS</td>
<td>Cigarette Smoke</td>
</tr>
<tr>
<td>csDMARD</td>
<td>Conventional Synthetic Disease-Modifying Anti Rheumatic Drug</td>
</tr>
<tr>
<td>DMARD</td>
<td>Disease-Modifying Anti Rheumatic Drug</td>
</tr>
<tr>
<td>EVALI</td>
<td>E-cigarette/vaping product use-associated lung injury</td>
</tr>
<tr>
<td>EURLAR</td>
<td>European League Against Rheumatism,</td>
</tr>
<tr>
<td>GC</td>
<td>Glucocorticoids</td>
</tr>
<tr>
<td>HLA</td>
<td>Human Leukocyte Antigen</td>
</tr>
<tr>
<td>IFA</td>
<td>Incomplete Freud’s Adjuvant</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobin</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>JAK</td>
<td>Januse Kinase</td>
</tr>
<tr>
<td>JAKi</td>
<td>Januse Kinase Inhibitor</td>
</tr>
<tr>
<td>KO</td>
<td>Knock Out (relates to mouse models)</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
</tr>
<tr>
<td>MP</td>
<td>Metacarpophalangeal Joints</td>
</tr>
<tr>
<td>MTX</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Non-Steroidal Anti-Inflammatory Drugs</td>
</tr>
<tr>
<td>OS</td>
<td>Oxidative Stress</td>
</tr>
<tr>
<td>PIP</td>
<td>Proximal Interphalangeal Joint</td>
</tr>
<tr>
<td>PG</td>
<td>Propylene Glycol</td>
</tr>
<tr>
<td>RA</td>
<td>Rheumatoid Arthritis</td>
</tr>
<tr>
<td>RF</td>
<td>Rheumatoid Factor</td>
</tr>
<tr>
<td>SE</td>
<td>Shared epitope</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal Transducer and Activator of Transcription</td>
</tr>
<tr>
<td>TC</td>
<td>Traditional Cigarettes</td>
</tr>
<tr>
<td>Tc</td>
<td>T-cell</td>
</tr>
<tr>
<td>Th</td>
<td>Helper T-cell</td>
</tr>
<tr>
<td>THC</td>
<td>Tetrahydrocannabinol</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>TNFi</td>
<td>Tumor Necrosis Factor Inhibitor</td>
</tr>
<tr>
<td>Treg</td>
<td>Regulatory T cell</td>
</tr>
<tr>
<td>tsDMARDs</td>
<td>Targeted Synthetic Disease-Modifying Anti Rheumatic Drug</td>
</tr>
<tr>
<td>VG</td>
<td>Vegetable Glycerin</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cell</td>
</tr>
</tbody>
</table>
INTRODUCTION

Rheumatoid arthritis is a chronic, inflammatory autoimmune disease which develops within the joints and can lead to life-threatening complications throughout the body. Currently, as with many autoimmune diseases, there is no cure for rheumatoid arthritis (RA). Pain management and immunosuppressive pharmaceuticals are the only options to which the over 23 million RA patients can turn.\(^1\) Approximately one percent of the adult population of the world experiences RA which makes it the most common form of rheumatic diseases.\(^1\) This equates to approximately 78,000,000 individuals. RA has no known cause, but current hypotheses note the potential roles of gene expression coinciding with environmental factors such as cigarette smoking (CS), obesity, age and gender.\(^2\)

Rheumatoid arthritis begins its path of destruction by attacking the synovium, then disseminate into the surrounding joints and bone causing massive tissue degradation.\(^3\) It first attacks the synovium- a flexible sac of fluid surrounding joints. Originating within small joints, RA then spreads to larger joints, and, in severe cases, certain organs.\(^1,2\) Some joints that are affected as this disease progresses include the wrists, elbows, and knees eventually leading to effects on organs such as the skin, eyes, heart, blood vessels, kidney, spleen and lungs.\(^2,3\) Fluid build-up, inflammation, deterioration, pain, and loss of joint mobility, are common symptoms of RA.\(^2\)

There are several forms of arthritis, therefore it is critical to note that RA is different from alternative forms of arthritis such as osteoarthritis because it affects the proximal interphalangeal (PIP) and metacarpophalangeal (MP) joints, rather than the distal joints of the hands and feet.\(^2\) This inflammation and destruction of the proximal joints leads to deformities such as the swan
neck and boutonniere, as shown in Figure 1 which lead to a severe decrease in quality of life and loss of mobility given their characteristic misshapenness, swelling and degradation.\textsuperscript{24}

Biologically, it is thought that rheumatoid arthritis is triggered by changes in the innate and adaptive immune systems which leads to autoantibodies such as Rheumatoid Factor (RF) and Anti-Citrullinated Protein Antibodies (ACPAs) being produced from B-cells. These autoantibodies are then allowed to circulate through the body thus eliciting a self-reactive response by cytotoxic T-cells (T\textsubscript{C}).\textsuperscript{2,5} These autoantibodies begin to attack the synovial membrane due to an error in differentiating self from foreign antigens which leads to inflammation and fluid build-up causing joint and cartilage erosion common with RA.\textsuperscript{5} A definitive causation of RA (prior to its measurable effects on the body) is still unknown, but it is thought to be affected by both an increase in genetic susceptibility and environmental factors, particularly cigarette smoking.\textsuperscript{2,4}

Cigarette smoking, as mentioned previously, is the number one environmental contributor to rheumatoid arthritis.\textsuperscript{6} Current data has established there is a strong correlation between CS exposure and RA development; specifically via the HLA-DRB1 gene responsible for autoantibodies RF and ACPA production.\textsuperscript{6} CS is associated with HLA-DRB1 activation, and therefore relates to the upregulation of autoantibody production.\textsuperscript{6} Notably, not all individuals who smoke traditional cigarettes experience an increase in autoantibody production- this is

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Degradation of RA joint. Depicts the notable deformations such as swan neck and boutonniere in the fingers caused by RA.\textsuperscript{24}}
\end{figure}
thought to be due to the duration and intensity with which the individual smoked, though duration is most critical for RA onset, rather than intensity alone. Regardless of autoantibody proliferation rates, however, CS is associated with an increased risk of RA development. Many studies have indicate the environmental and genetic impacts of CS on RA, however, few have concerned the effects of e-vapor.

E-vapor, otherwise known as vaping, is a fairly new form of e-cigarette popular amongst adolescents and young adults. Over 16 million adolescents vape within the United States since the beginning of 2020 which is a drastic increase from 2018 statistics listing 1.3 million adolescents actively vaping. This robust market offers many options to consumers in order to vape based on their individual preferences. For instance, vaping machinery varies greatly in design and purpose- including but not limited to ohm power, heat capacity of the coils, density of vapor expelled, flavoring, and the sizes of the modules. Due to the large variability amongst the types of vape available, few studies have been conduction on its health implications, either negative or positive. With the large array of vape modules on the market, standardizing experimentation has posed as an obstacle within laboratory settings. The Center for Disease Control states that smokers who utilize vaping as a way to quit smoking in the traditional manner, due to the hypothetical decreased levels of carcinogens in vaping products. However, opposing studies hypothesize that vaping is just as severe to one’s health as CS, if not more. This stark contrast of data epitomizes the current understanding of vaping. Ultimately, there is not scientific, nor popular opinion, consensus on the effects of vaping. In fact, most of the literature available on the subject are based on the legislation surrounding how vape companies target minors via advertising, as well as public perception of vape. The studies that have been released concerning vape and human health, indicate vaping leads to high rates of reactive
oxygen species in the body, as well as negative repercussions on lung health.\textsuperscript{9,10} The long-term effects of vape have yet to be uncovered. Therefore, there is an immense need for studies focused on the complexity of e-vapor. Since vaping is tremendously popular amongst youth and young adults, the need has quickly arisen to study longitudinal effects of vaping. Given the contributions to RA CS has, there may prove to be a strong correlation between vape and the onset of rheumatoid arthritis later in life. Studies must be pursued in order to ascertain the possible effects of vaping on RA to save a future generation from what may be a fairly avoidable and severe autoimmune disease.
METHODOLOGY

Research Methodology

Since the data concerning the subject matter of Rheumatoid Arthritis and cigarette smoking is vast, but the information pertaining to RA and e-vapor is sparse, the most effective route of methodology is an extensive review of the literature. Databases such as PubMed, EBSCO, Academic Search Complete and others were utilized in order to closely study the research of those who have paved the way in this content area. Furthermore, a proposed laboratory experiment concerning the effects of e-vapor on rheumatoid arthritis mouse models is disclosed beginning on page 40. It is critical to note that though this experiment is theoretical, it is believed to be an effective summation and comparison of the current data on cigarette smoke, e-vapor and their effects on the potential induction of rheumatoid arthritis and its pathogenicity, particularly in adolescents.
The Immune System

The immune system is a highly efficient and complex network present throughout the human body. This elaborate system of checks and balances for the overall health of the body is made possible by the intricate relationships, chain reactions and signaling abilities between millions of immune cells. Rheumatoid arthritis is characterized by the malfunction of said immune cells. RA occurs when there is a change from immune cells being protectors of nonpathogenic tissue to agitators against the body, in particular cells that make up the synovial membranes surrounding joints. This phenomena is thought to occur due to autoantibody B lymphocytes in conjunction with T-cells (T-lymphocytes) becoming proinflammatory effector cells instead of regulatory T-cells (T$_{reg}$) thus causing a chain reaction response in the immune system. T$_{reg}$ cells function to suppress the responses of other T-cell subsets (such as proinflammatory T$_{H1}$ and T$_{H2}$ cells), antigen presenting cells and B-lymphocytes. These autoreactive (self-attacking) T-cells partner with autoantibodies released from B-cells thus eliciting a destructive immune response leading to joint degradation, inflammation and the eventual replacement of healthy flexible tissue with dense fibrous tissue. Before the RA pathogenicity can be fully understood, however, one must comprehend the key players in a healthy immune system.

Antibodies

Antibodies, otherwise known as immunoglobulins (Ig), are the linchpin to the immune system. Without these proteins, the body would be largely unable to recognize foreign pathogens such as bacteria, viruses and foreign entities. There are two broad types of lymphocytes immune cells, T-lymphocytes, also called T-cells, and B-lymphocytes known as B-cells.
cells generate billions of unique forms of themselves and antibodies that correlate with their characteristics in order to ward of infection. This diversity is crucial to the adaptive immune system which learns to identify specific pathogens by experiencing multiple exposures to said molecules, thus creating memory within the immune system.

There are five broad categories of immunoglobulins, IgA, IgD, IgE, IgG and IgM. Each type of Ig correlates with one specific type of antigen-binding site. IgM is known for its role on naïve B-cells which is further explained in the following paragraphs. IgA is common within the mucosal lining and activates mucosal tissue responses to pathogens. IgE activate granulocytes (such as mast cells, eosinophils and basophils) and primarily respond to allergens or parasites within the body. IgD is not well understood but potentially assists in process of B cell maturation. IgG is famous for its role in binding to Fcγ receptors on T cells which are then activated to respond and assist mature B-cells (also known as effector or plasma cells). IgG is an opsonin which is a biochemical marker that signals for phagocytosis. Its role is critical to the negative effects of RA, but it may also play a role in the prevention or treatment of rheumatoid arthritis. IgG is the most noteworthy Ig for RA, and while the other antibodies are significant within the body they have shown little direct correlation with RA for the purpose of the discussion on hand.

Antibodies have exclusive relationships with antigens. A specific antibody will only bind to a specific antigen. Antigens, or molecules that cause an immune response, can range anywhere from a sliver of wood in an index finger to cancerous cells, and nearly anything in between. In ideal situations, the immune system is able to detect, label and bind with each specific enemy it faces in order to quickly and effectively terminate the threat.
Each B-cell generates an antibody with one type of receptor for a specific antigen.\textsuperscript{11} Prior to releasing an antibody army against pathogens though, each naïve B-cell within the bone marrow must undergo a maturation process so it learns to recognize foreign invaders from healthy, nonpathogenic, “self,” cells.\textsuperscript{11} IgMs are presented on the B-cell surface as a way for the immature B-cell to identify a new threat from a variety of cells it faces and then allows it to signal for specific immune responses.\textsuperscript{11} Upon binding with a foreign substance, as a B-cell generates its first antibodies aside from IgM, the antibodies are inserted into the plasma membrane.\textsuperscript{11} With $10^5$ receptors on a B-cell, the maturing B-cell with its respective antibodies is able to quickly signal through intracellular pathways when an antigen binds to any of its antigen-binding sites.\textsuperscript{11} Once an antigen is bound to the immature naïve B-cell, the B-cell multiplies and differentiates into an antibody-secreting effector cell with the assistance of a helper T-cell (Th) in Figure 2.\textsuperscript{11}

\textit{Figure 2: Activation and Class-Switching of B-cells. This indicates the complex relationship antigen presenting cells, T-helper cells, active T cells, and B-cells have with one another in order to elicit a healthy immune response.}\textsuperscript{12}
Specific cytokines are then released in response to the B-cell/T-cell partnership. Once the B-cell has matured, the antibodies it makes are secreted rather than bound to the plasma membrane since the B-cell has learned to recognize pathogen from self-cells and is now considered an active plasma cell. If a B-cell is unable to differentiate between self and pathogenic material, it is destroyed or deactivated unless it evades those actions.

**Antibody Structure**

B-cells generate antibodies. Antibodies are able to bind to antigens due to their unique molecular structure and binding regions. Each antibody is a “Y”-shape consisting of two heavy polypeptide chains and two light chains, as shown in Figure 3.

The heavy chain gives an antibody a name— one of any of the five classes mentioned prior (IgA, IgD, IgE etc.). The hinge regions between the “Y” arms and the tail (Fc region) of the “Y” are uniquely capable of creating more or less space between the antigen binding sites in order to optimize binding affinity.

The antigen binding sites are at either end of the “Y” arms and between the N-terminal regions of one light chain and one heavy chain. Although there is a pair(s) of antigen binding sites, it

---

**Figure 3: Antibody Structure.** Antibodies consist of two light chain and two heavy chain regions. Additionally, the ends of the antibody where the antigen binding site is located are known as variable regions while the tail of the antibody is the constant region.
is necessary to note this does not change the one-to-one binding relationship of an antibody to a specific antigen. The stem of the “Y” is the portion of an antibody other immune cells, like Th cells, read in order to determine how they should respond to the antigen the antibody is holding.

**Complement**

The immune system functions in two broad but intertwined categories: innate and adaptive. The complement system is most often considered a product of the innate immune system. It is a way for antibodies, a component of the adaptive immune system, and phagocytes such as macrophages, monocytes and granulocytes to enhance their collaboration. This partnership allows them to rid microbes and damaged cells from the body. Within this process four major functions are executed: lysis of infectious organisms, activation of inflammation, opsonization and immune clearance. Lysis and apoptosis is often achieved by phagocytes, short lived neutrophils, and longer living monocytes or macrophages which “digest” cell matter. The act of degradation via any of these cells, depending on the signals received, can quickly lead to inflammation. Inflammation by definition is the accumulation of fluid and cell matter caused by blood vessel dilation. This is a positive result of the body attempting to fight off an attack, thus signaling for an influx in white blood cells however, when inflammation is chronic it leads to serious repercussions— such is the case in RA. Opsonization is a biochemical marker which is brought on most frequently by IgG on plasma cells and indicates the need for antigen destruction by phagocytosis.

In order for cells to recognize the need for phagocytosis, there must be T-cell activation, which is triggered by antigen presentation. The adaptive and innate immune systems work via B-cells and other antigen presenting cells (APCs) such as macrophages and dendritic cells (DC),
respectively expressing class II MHC molecules while all nucleated cells express MHC class I. MHC I signifies two main roles. Firstly, it exclusively signals for cytotoxic T-cells that kill threats on contact. Secondly, MHCI is the indication to immune cells that the cell it is on is “self,” therefore cells that have MHCI on their surfaces are not destroyed by natural killers (NKs). Major histocompatibility complex (MHC II) is required for T cells to read an antigen. This differs from B cells which can bind directly to a pathogen, in that rather than displaying a whole antigen on its surface, MHCII display small peptides of an antigen on the cell surface for T-cell receptors. MHC II recruits helper T-cells which positively assist B-cells by aiding in their release of antibodies. In autoimmune diseases where MHC II malfunctions, only MHC I is able to signal which leads to extensive cellular death brought on by the excessive presence of cytotoxic CD8+ T-cells.

As opposed to cytotoxic T-cells, when helper T-cells are activated they divide and secrete cytokines that can regulate and assist in the immune response. They are able to signal for white blood cells to respond to the infected area, thus increasing symptoms of an immune response, but not exclusively calling for immediate phagocytosis of threatening cells. Research has indicated that helper T-cells as well as effector cytotoxic (T\textsubscript{C}) act within the synovium of joints of RA patients. In the synovium of RA patients, Th and T\textsubscript{C} cells signal for increased cellular response from immune cells, such as macrophages, B-cells and cytotoxic T cells, and destroy healthy cells perceived to be pathogenic, respectively. Once cytotoxic T-cells are activated, they are effector cells which target and kill any other cell that has the same pathogen within it.

Normally, the body screens for self-reactive T-cells during their development in the thymus. However, self-reactive T-cells either by-pass checkpoints during their maturation process or mutate later to become self-reactive. Self-reactive T-cells are able to independently
bind strongly to self MHC complexes rather than requiring the assistance of CD4/8 co-effectors.\textsuperscript{15} Since the T-cell does not require a co-effector, it is able to bind to any cell that is displaying self MHC, rather than self MHC that is simultaneously displaying a foreign pathogen.\textsuperscript{15} This upheaval in the T-cell binding affinity leads to a heightened immune response due to the increase in apoptosis.\textsuperscript{15} Chronic inflammation caused by the cytokines released, as well as the flood of white blood cells signaled to the area due to the cytotoxic cell destruction, leads to the symptoms of RA.\textsuperscript{13} A clear route from acute to chronic inflammation has not yet been discovered, but chronic inflammation is associated with an increase in macrophages and lymphocytes to the site of infection.\textsuperscript{17} Long-term inflammation signals the body to have increased blood flow/vasodilation which naturally increases the migration of white blood cells to the area where antibodies are signaling for a response. This response becomes a vicious cycle that perpetuates the devastation of RA.\textsuperscript{18}

Healthy immune response ordinarily begins with neutrophils because they are able to signal for inflammatory response, but these cells quickly die to prevent prolonged signaling.\textsuperscript{18} In rheumatoid arthritis, due to persistent inflammatory signals, longer living white blood cells (WBC) such as macrophages and lymphocytes have to respond to the inflammation and cell deaths since the neutrophil population becomes depleted.\textsuperscript{18} The normally healthy process of a quick and efficient response to pathogens instead leads to long-term inflammation and degradation due to the prolonged presence of macrophages, lymphocytes and the cells they signal (B and T-cells).\textsuperscript{18} In turn, degradation leads to the replacement of healthy tissue around the bones to be replaced with much stiffer connective tissue built by fibroblasts since the synovium is degrade to such large degrees.\textsuperscript{18}

\textit{Cytokines}
Cytokines are signaling molecules that act as regulators of temperature, inflammation, neutrophil mobilization and many other responses in the immune system. Early RA is thought to be affected by eleven different cytokines, including cytokine tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6), although this data is tentative. TNF and IL-6 are both pro-inflammatory cytokines which by definition increase inflammatory response and increase osteoclast differentiation in the joints, specifically. Together, these two cytokines simultaneously increase pressure within the synovium around joints and increase the population of cells that degrade bone. Also key to rheumatoid arthritis are T-helper 17 (Th17) cells. Th17 cells produce cytokines interleukin-17A (IL-17A), IL-17F and IL-22. IL-17A and IL-17F are known to recruit and activate neutrophils and stimulate other cells to produce inflammatory cytokines such as IL-6. This combination of Th17 cell subtypes is thought to be a cause to the chronic inflammation within the joints of RA patients.

**Autoantibodies**

Autoreactive T-cells interact with autoantibodies released from self-reactive B-cells. Autoantibodies are antibodies that have become self-reacting, rather than self-protecting. Ordinarily, autoantibodies are only found in minute amounts as IgM molecules, however, large amounts of autoantibodies cause detriment to the body. Autoantibodies that undergo mutations are no longer non-antigenic, therefore they trigger defensive immune reactions against self-cells. The chemical trigger which leads to the change from healthy antibodies from B-cells to autoantibodies is largely undiscovered. Alberts, Johnson and team showed great promise in their recent study indicating how Rho, a small GTPase, may signal for negative changes to B and T-cell development, activation proliferation differentiation and migration. Regardless,
mutation(s) within IgM antibodies become IgG autoantibodies—known to be more widespread and have specific adhesion properties compared to healthy IgM which do not have specific adhesion properties. This change thus leads to a broader ability to inaccurately bind to self-cells and elicit an unnatural and detrimental immune response. ACPA and RF are two autoantibodies commonly present in preclinical disease onset. As mentioned within the Biomarkers portion of this discussion, these autoantibodies have been utilized as early indicators that RA may develop, and its severity may be predicted. Also, the increased production of these autoantibodies is strongly correlated with the environmental factor of cigarette smoking. Therefore, recent literature has begun to explore the possible genetic pathways affected by cigarette smoke that may lead to this increased proliferation of autoantibodies.
Rheumatoid Arthritis

*Rheumatoid Arthritis*

Rheumatoid arthritis is a chronic, inflammatory autoimmune disease which develops within the joints and can lead to life-threatening complications throughout the body. Currently, as with many autoimmune diseases, there is no cure for rheumatoid arthritis (RA). Pain management and immunosuppressive pharmaceuticals are the only options to which the over 23 million people affected by RA can turn.\(^1\) Approximately two percent of the adult population of the world experiences RA which makes it the most common form of rheumatic diseases.\(^1\) RA has no known cause, but current hypotheses note the potential roles of gene expression coinciding with environmental factors such as cigarette smoking (CS), obesity, age and gender.\(^2\)

Rheumatoid arthritis begins its path of destruction by attacking the synovium, then creeps its way into the surrounding joints and bone causing massive tissue degradation.\(^3\) It first attacks the synovium- a flexible sac of fluid surrounding joints. Originating within small joints, RA then spreads to larger joints, and, in severe cases, certain organs.\(^1,2\) Some joints that are affected as this disease progresses include the wrists, elbows, and knees eventually leading to effects on organs such as the skin, eyes, heart, blood vessels, kidney, spleen and lungs.\(^2,3\) Fluid build-up, inflammation, deterioration, pain, and loss of joint mobility, are common symptoms of RA.\(^2\) There are several forms of arthritis, therefore it is critical to note that RA is different from alternative forms of arthritis such as osteoarthritis because it affects the proximal interphalangeal (PIP) and metacarpophalangeal (MP) joints, rather than the distal joints of the hands and feet.\(^2\) This inflammation and destruction of the proximal joints leads to deformities such as the swan neck and boutonniere, as shown in Figure 4 which lead to a severe decrease in quality of life and
loss of mobility given their characteristic misshapenness, swelling and degradation.  

Biologically, it is thought that rheumatoid arthritis is triggered by changes in the innate and adaptive immune systems which leads to autoantibodies such as Rheumatoid Factor (RF) and Anti-Citrullinated Protein Antibodies (ACPAs) being produced from B-cells. These autoantibodies are then allowed to circulate through the body thus eliciting a self-reactive response by cytotoxic T-cells ($T_C$). These autoantibodies begin to attack the synovial membrane due to an error in differentiating self from foreign antigens which leads to inflammation and fluid build-up causing joint and cartilage erosion common with RA. A definitive causation of RA (prior to its measurable effects on the body) is still unknown, but it is thought to be affected by both an increase in genetic susceptibility and environmental factors, particularly cigarette smoking.

Etiology

The etiology of rheumatoid arthritis is still unknown, however, there are several factors that lead to an increased risk of developing RA. Gender, age, obesity, genetic susceptibility, environmental factors and biomarkers all can indicate vulnerability to developing this autoimmune disease. Research has unanimously concluded that the most vulnerable population by far is middle-age women— notably, there seems to be few differences in susceptibility when the ethnicities of middle-age women are considered.
Beyond one’s sex increasing the risk of RA, there are many studies which support the potential for familial inheritance to play a role in the susceptibility of a patient to develop rheumatoid arthritis.\textsuperscript{1} Several genes have been identified as possible ties to RA. Major Histocompatibility Complex (MHC) genes, which consist of three separate classes of “HLA-X,” are thought to be one of the genomic indicators of RA.\textsuperscript{1} The HLA-DRB1 gene is one of the Human Leukocyte Antigen (HLA) gene complexes whose role is to encode for MHC class II antigen-presenting molecules.\textsuperscript{6} MHC molecules, as described in the \textit{Immune System} section, are antigen-presenting molecules responsible for the relationship immune cells have with peptides on the outside of antigen presenting cells (APCs).\textsuperscript{5} If the protein made by HLA-DRB1 holds a peptide that the immune cells do not recognize it, they signal for an inflammatory response in order to rid the body of the foreign antigen.\textsuperscript{7} This cascade of events beginning from the MHC gene is therefore critical in anti-citrullinated protein antibody (ACPA) positive patients because these autoantibodies are unrecognized by the body and trigger an inflammatory immune response.\textsuperscript{1} The role of gene expression in RA pathology is described further in the section \textit{Gene Expression} for RA.

Biomarkers are measurable indicators of normal healthy processes or pathogenic operations that can be used to detect rheumatoid arthritis.\textsuperscript{7} Two biomarkers, anti-citrullinated protein antibodies (ACPAs), as mentioned previously, and rheumatoid factor (RF) play a significant role in determining the probability of developing RA and its severity.\textsuperscript{7} Over 75% of RA patients express these biomarkers, hence making them a lead indication for RA.\textsuperscript{1} High levels of RF, in particular, indicate the risk for developing aggressive rheumatoid arthritis that greatly limits joint functionality.\textsuperscript{7} ACPAs develop as ACPA-positive or negative, the positive indicating a patient may develop more bone erosion and severe disease progression in comparison to
ACPA-negative patients.\textsuperscript{7} ACPAs are perpetuated by environmental factors such as smoking.\textsuperscript{7} RA proves difficult to treat since individual patients experience varying levels of inflammation, locations of RA and severity of pain thus the analysis of biomarkers such as autoantibodies, like ACPA and RF, help health care providers create treatment plans and predict the success of said treatments for each individual case of RA.\textsuperscript{2,13} Due to the very nature of biomarkers, however, they alone cannot be taken as proof for susceptibility to the autoimmune disease RA as they indicate other autoimmune diseases or infections as well.\textsuperscript{7} Nonetheless, research has indicated that an increase in autoantibodies ACPA and RF are highly indicative of future RA development.\textsuperscript{2}

A review published by C. Croia et. al. disclosed a list of the most likely environmental factors that contribute to RA, as described in any 2019 articles published on RA. Amongst the list were factors such as smoking, dietary habits, obesity, infections and others.\textsuperscript{1} Obesity was shown to correlate with increased risks of RA for women.\textsuperscript{1} This correlation is thought to be caused by adipokines such as leptin which are cytokines released from adipose tissue.\textsuperscript{1} Leptin may, in fact, be a player in inflammatory response and bone erosion.\textsuperscript{8} While obesity has been shown to correlate with an increased risk of RA, cigarette smoking nearly guarantees development of RA.\textsuperscript{1} Studies have indicated that cigarette smoking is the most pertinent external environmental factor to prompt RA.\textsuperscript{1} In fact, mouse models exposed to cigarette smoke (CS) condensate expressed arthritis one day after exposure.\textsuperscript{1} However, the mechanisms effected by CS are uncertain. Nonetheless, there appears to be a strong positive correlation between smoking and the risk for rheumatoid arthritis. The list of factors that contribute to the potential for one to develop rheumatoid arthritis is extensive however, so there is still great debate amongst the scientific community as to the causes and foolproof indicators for RA.\textsuperscript{15}
Rheumatoid arthritis is largely considered a genome-based and environmentally influenced autoimmune disease. Current studies discovered the HLA-D1 gene clearly partakes in RA morbidity, however its influence on the disease is yet to be fully understood. Genome-wide association studies (GWAS) have indicated there are over 100 loci (genes) that are in association with an increased risk of RA.

HLA, human leukocyte antigens, are MHC class II surface receptors which are encoded by the HLA complex present on the sixth chromosome in humans. Three classes of HLA exist, and they are all glycoproteins bound to the cell surface. Class I HLA and Class III assist in the process of peptide presentation inside the cell and play a role in the complement system activation, respectively. HLA class II is the lynchpin for displaying peptides on the outer surface of APCs. Without HLA class II it would be an anomaly for T helper CD4+ T cells to be activated, thus preventing a well-rounded immune response.

ACPA, an autoantibody and indicator for the onset of RA, is tested in potential RA patients and determined as either present or absent. Prior research once indicated there was a difference in genetic susceptibility for ACPA-positive patients as compared to ACPA-negative, but this has since been disproven showing no difference in the heritability of RA when studied in twins. The severity of disease manifestation, however is indicated by a ACPA-positive test. Citrullination, the “C” in ACPA, is a posttranslational modification that occurs when a charged arginine is replaced by a neutral citrulline on self-proteins (proteins which ordinarily abide in the body). The variable region of these citrullinated antibodies have unique N-linked glycans (especially on ACPA-IgG) which is likely the physical difference between ACPAs and nonpathogenic antibodies. Specifically generated by citrullinated B cells, ACPAs react with
citrullinated autoantigens within the blood and articular cartilage. For instance, citrullinated fibrinogen is normally present within the blood plasma, however during inflammation events the levels of citrullinated fibrinogen increases nearly threefold. This autoantigen glycoprotein complexes with ACPA to stimulate Fcγ receptors on macrophages. Fcγ receptors then signal for the release of TNF-α, a proinflammatory cytokine, thus eliciting an immune response. Therefore, ACPA leads to an intensified and autoreactive response, all beginning with the expression of the HLA-DRB1 gene, as depicted in Figure 5.

Figure 5: A detailed analysis of how HLA-DRB1 gene expression interacts with the complex autoimmune response pertaining to RA.
HLA Genes

HLA is a relevant gene to RA pathogenicity because of its relationship with ACPAs. HLA expression is similar in both young-onset and normal RA, which indicates its role is of unique importance regardless of age. HLA-DRB1 is associated with positive ACPA RA, in particular. There has long been a hypothesis concerning three amino acid sequences [70QRRAA74, 70RRRAA74 or 70QKRAA74 (where R is Arginine, A is Alanine and K is Lysine)] which are commonly referred to as the “shared epitope” (SE). The presence of these SE in the HLA-DRB1 gene is thought to allow for self-antigens to be presented to T lymphocytes. Approximately 75% of patients with RA have the HLA-DRB1 SE allele. Indeed, RA development is three to five times higher in patients with the SE allele than in those who do not. There are several loci within the HLA class II region that have been shown to play a role in RA: HLA-DP, HLA-DQ, and HLA-DR. HLA-DRB1 shared epitope is also strongly associated with ACPA-positive RA and the overall presence of autoantibodies. HLA-DRB1 SE also corresponds with increased mortality, though interestingly SE is most common in men, not women which is the population group which is normally most susceptible to RA. Perhaps one of the most interesting findings concerning HLA-DRB1 SE is its relationship with cigarette smoking (CS). This environmental factor, coupled with HLA-DRB1 SE, positively correlates with an increase in autoantibody production in RA. Specifically, there are six exons on the HLA-DRB1 gene. (This is the location on a gene where the information for coding a protein or peptide is stored.) Exon 2 is where the antigen recognition site for coding antigen recognition is housed, therefore Exon 2 in the HLA-DRB1 gene is crucial to the potential changes in immune recognition that leads to RA.
HLA-DRB1 is not the only gene thought to influence or be influenced by RA pathogenesis.\(^1\) The list is quite exhaustive (over 100 genes have been identified), but there is a handful of genes which are more noteworthy than the others.\(^1\) CD97, FYB, CXCL1, IKBKE, CCR1 are genes which play a direct role in immune response.\(^1\) CD97, CXCL1, C3 AR1, CCR1, and LYZ induce inflammatory responses, and C3 AR1, CCR1, PLN, CCL19, PPT1 are involved in homeostasis.\(^1\) Some of these genes, such as CXCL6 and CXCL1, are part of the same C-X-C motif rather than two entirely distinct genes.\(^1\) Each RA-related gene plays an intricate role in RA as expressed in the supporting literature extensively.\(^1,18,19\) CXCL1, for instance, is known to powerfully attract neutrophils in the synovial tissue and fluid of RA.\(^1\) This heavy attraction is triggered by TNF-\(\alpha\) or IL-1\(\beta\) both being key players in RA.\(^1\) Studies such as the microarray depicted in *Supplemental Data 1* have been shown as an effective gene analysis tool.\(^20\) Indeed, much more research is needed in the area of gene expression for RA considering the vast implications even a single gene may have on the various pathways involved in RA pathogenicity.\(^18,20\)
Current Treatments

_Treatment Course of Action_

Symptom-based treatment options are available for patients with RA. Since each case of RA can vary in pain intensity, bone degradation and an individual’s response to pharmaceuticals, there are a slew of treatment options. Nonsteroidal anti-inflammatory drugs (NSAIDS), corticosteroid medications, and various forms of disease-modifying antirheumatic drug (DMARDs) agents are the most common forms of drugs used to combat the symptoms. Since most patients experience joint damage as early as two years into their initial diagnosis, early treatment in the beginning months of diagnosis is of the utmost importance. Physicians prescribe drugs depending on the severity of each case in order to control inflammation, and potentially achieve remission for their patients if the treatment is prompt and effective.

In addition to drug intervention is the importance for patients to be educated about the importance of exercise and physical therapy. Weight bearing exercises have been shown to particularly aid in the retardation of RA symptom onset. According to studies of biomarkers, RA is perpetuated by factors such as obesity and smoking, so patients with RA who take preventative measures against these conditions and habits will only benefit in the long-run for their disease remission outcome.

Unfortunately, due to the autoimmune nature of RA and the severity of its progression, as well as the intensity of immunosuppressing drugs, many patients are backed into a metaphorical corner. They are crushed by the trifecta of severe pharmaceutical side effects, grating pain or living in a constant state of immunosuppression. It is for these reasons that one of the greatest current struggles in treating RA is the ineffectiveness of drugs for long-term remission. Given
RA has both genetic and environmental factors which contribute to its development, it is imperative to assist patients in taking preventative measures against this disease.

The European League Against Rheumatism (EULAR) has compiled a three phase, multifaceted set of 10 recommendations for how to treat RA as well as a pointed, target-to-target approach for optimal patient outcome. Supplemental Data 3 depicts said recommendations. The treatment process is split apart in three phases, each dependent on how a patient responds to the selected pharmaceuticals and if a target outcome has been achieved. Methotrexate, an immunosuppressive antitemtabolite DMARD drug, is often suggested as the initial treatment for RA, however if a patient reacts poorly to methotrexate or other conventional synthetic DMARDs (csDMARDs), alternative DMARDs can be prescribed. Either of these treatments can be paired with short term, low dose glucocorticoids. If these combinations fail to aid in decreasing inflammation and pain after six months, a patient enters phase II. Characterized by the addition of biologics, otherwise known as biologic DMARDs, treatments with a targeted DMARD “TNF-inhibitor” or an “IL-6 inhibitor” in this phase heightens the intensity of drugs used to fight progressing RA symptoms. This pharmacological approach was discovered largely by analyzing the effects of HLA-DRB1 SE in RA patients. If a patient moves into phase III recommendations, alternative biologics are prescribed. A Janus kinase inhibitor (JAK-inhibitor) is a recent discovery and last resort for treatment options. Importantly, if an individual experiences positive results from any of the treatment in the various phases, the drug regimen is not discontinued, rather it is repeated indefinitely in order to maintain anti-inflammatory effects, decrease pain and potentially increase quality of life. The hurdle for long-term remission in so many RA cases however is the ability of the immune system to create antibodies against the biologics introduced to it.
Glucocorticoids

During the early disease state of RA, glucocorticoids (GC) may be utilized in order to control inflammation, swelling and relieve pain quickly. GCs are a subgroup of steroids, therefore, their use is most effective for short term, 3-4 month, periods and are not recommended to be used longer than that time frame due to their harsh side effects and possible comorbidities. Therefore, GCs can be replaced by or paired with DMARDs for long-term inflammation dampening. GCs are effective against inflammation due to their mechanisms of action by inhibiting B and T cells. GCs suppress proinflammatory molecules in a process known as transrepression. The opposing but simultaneous action of GCs is transactivating anti-inflammatory molecules. Transactivation is thought to cause adverse effects within the body during chronic use of GCs. A few of the adverse side effects include weight gain, fat redistribution such as a buffalo hump as shown in Figure 7 (buffalo hump source), osteoporosis, fractures, cardiovascular effects such as atrial fibrillation, flutter, and heart failure, increased risk of infection, and lastly, hyperglycemia. Patients are more likely to experience serious

Figure 6: Buffalo hump, or the accumulation of adipose tissue on the neck and trunk of a male rheumatoid arthritis patient. (View is both lateral and posterior).
effects such as these if GC use is prolonged, so they are rarely prescribed on a long-term treatment regimen.\textsuperscript{22,23} Disease-modifying antirheumatic drugs can therefore be prescribed in conjunction with or in place of glucocorticoids.\textsuperscript{22,25}

\textit{Methotrexate}

Methotrexate (MTX) and other conventional DMARDs dampen the inflammatory response via several modes of action including downregulating the production of TNF.\textsuperscript{22} Methotrexate is one of the most commonly prescribed conventional synthetic DMARD (csDMARD) though its high dose related side-effects are severe and can be life threatening.\textsuperscript{22,25} The milder side effects of low-dose MTX are WBC and platelet deficiency, headaches, gastrointestinal complications, but many of these can be subdued by folate supplementation.\textsuperscript{22} Folate supplementation is necessary because MTX treatment causes the body to dispel it at higher rates than in normal function.\textsuperscript{26} This affects a person as a whole because folate helps prevent anemia, aids in tissue growth and cell action and is all-around essential to normal bodily functioning.\textsuperscript{26} MTX can be replaced with other DMARDs such as leflunomide, hydroxychloroquine or sulfasalazine if a patient reacts poorly to MTX; these drugs express similar success to MTX.\textsuperscript{22}

The process of determining which drug is most effective for an individual can be long and grueling. The effects of MTX are often not experienced until 12 weeks into consistent oral ingestion of the drug either once a day or weekly depending on recommendations.\textsuperscript{22} After 24 weeks of drug treatment, then a decision can be made as to whether or not MTX is assisting in remission of RA.\textsuperscript{22} This drawn-out effect thus can leave many patients frustrated with their treatment and lead them to discontinue the drug unsafely.\textsuperscript{22}

\textit{Tumor Necrosis Factor-Alpha Inhibitors}
Tumor Necrosis Factor-Alpha Inhibitor (TNFi) is unique compared to GCs because they are antibodies which target tumor necrosis factor (TNF) within the immune system.\textsuperscript{22,27} TNFi is a biologic meaning it is composed of large antibodies which were derived from living cells in a laboratory setting.\textsuperscript{28} Biologics can be large or small molecules and are not always antibodies, regardless, they always target either a specific genotype or protein receptor.\textsuperscript{28} In the case of TNFi, it targets the latter.\textsuperscript{28} Recall, TNF signals for proinflammatory cytokine production, such as IL-6, and inhibits T_{reg} cells.\textsuperscript{19} There are currently five TNFi available for treatment and while each can be utilized to combat various diseases all can be applied to RA treatment, the most common of which is Infliximab.\textsuperscript{22,27,29} TNFi acts to hinder TNF by binding to its correlating receptors present on nearly all types of cells other than erythrocytes.\textsuperscript{27} The two receptors to which TNF normally binds are so1TNF and tmTNF which signal for inflammatory response and increased sensitivity to infection, respectively.\textsuperscript{27,29} The issue with TNFi is its non-selectively.\textsuperscript{29} TNFi blocks both the receptors TNF normally binds to which is beneficial for anti-inflammatory effects but of detriment for the ability of the immune system to fight infection.\textsuperscript{29}

**IL-6 Inhibitors**

An IL-6 inhibitor binds more specifically than TNFi’s.\textsuperscript{22} This inhibitor prevents the actions of IL-6, a cytokine abundant in the synovium of RA patients and known for its proinflammatory activation.\textsuperscript{22,30} IL-6 may contributes to B-cell differentiation in turn producing autoantibodies such as RF and ACPA.\textsuperscript{30} IL-6 also induces differentiation of T-cells into IL-17 secreting Th17 T helper cells thus preventing T_{reg} divergence.\textsuperscript{31} To add insult to injury, quite literally, IL-6 may encourage synovial fibroblast changes and osteoclast activation leading to further degradation and annihilation of cartilage and bone matter.\textsuperscript{30}
IL-6 inhibitors are most commonly monoclonal (synthetically produced antibodies) that bind to both membrane-bound and soluble IL-6 receptors, hence blocking the option for the cell to signal for others hence preventing an increase in inflammatory response.\textsuperscript{17} The common prescription IL-6 inhibitor is Tocilizumab.\textsuperscript{30(\textsuperscript{p6})} IL-6 continue to be normally produced by the innate immune system cells such as macrophages, and neutrophils, as well as adaptive immune B-cells, but they are rendered inactive when an IL-6 inhibitor binds to its receptors.\textsuperscript{32} Unlike other inflammatory diseases whose inflammation is characterized by TNF, IL-6 is thought to indicate inflammatory response and the pathogenesis of RA.\textsuperscript{32} In terms of treatment with IL-6 inhibitors, a patient may be able to receive intravenous drip infusion once every four weeks with dosage depending on clinical response. The benefit of this treatment is that its impact, whether null or positive, is observable more quickly than TNFi or MTX treatment options.\textsuperscript{30,32}

\textit{JAK-Inhibitors}

The final resort for many RA courses of action is a Janus-Kinase Inhibitor. Drugs such as tofacitinib are JAK-inhibitors, targeted, synthetic DMARDS (tsDMARDS) with a mechanism of action that blocks tyrosine kinase.\textsuperscript{22} JAK are tyrosine kinases present in the cytoplasm.\textsuperscript{22} There are 90 forms of Jaks which fall into four categories: Tyk2, Jak1, Jak2 and Jak3.\textsuperscript{33} The JAKs pertinent to RA play an intricate part in signal transduction to the nucleus from interleukins and act as downstream mediators for pro-inflammatory cytokines such as IL-6.\textsuperscript{22,33} This downstream effect is possible because when JAK and cytokines bind, phosphorylation of signal transducer and activator of transcription (STAT) molecules occurs (hence the “kinase” characteristic of JAK).\textsuperscript{22} The phosphorylated STATs then dimerize and can enter the nucleus leading to increased signaling for inflammatory response- a system that is expressed in Figure 7 (Janus Kinase inhibitors source).\textsuperscript{22} The Jak/STAT pathway is often utilized by cytokines to exert their effects
and therefore JAK has become a promising target for recent therapeutics. Concerning RA, the two available drugs target JAK1/JAK2 or Jak1/JAK3, specifically. Tofacitinib blocks Jak1 and Jak2 thus preventing Th17 cells from generating. This action prevents the production of a number of pro-inflammatory cytokines which would otherwise be activated by the Th17.

Figure 7: JAK-Inhibition Mechanism. Cytokines bind to receptors, thus eliciting an intracellular phosphorylation of the tyrosine kinase receptor. STATs are signaled for, phosphorylated by JAKs and as a result become dimers. STATs play a large role in regulating gene transcription, therefore, JAK inhibitors target the initial phosphorylation of STATs via JAK. Listed are a number of known JAK-inhibiting drugs.
Cigarette Smoke and E-Vapor

*Cigarette Smoke*

Cigarette smoke (CS) has long been considered the leading environmental factor to contribute to RA pathogenesis. Studies indicate that smoking one pack of cigarettes a year, which is equivalent to 20 cigarettes, increases the likelihood of contracting RA by 26 percent; this susceptibility only increasing as the number of cigarettes smoked throughout a person’s lifetime increases.

Two of the most critical factors for CS exposure as it pertains to RA development are intensity and duration, though duration is most impactful. For instance, an individual who smokes throughout the course of his/her life is far more likely to be diagnosed with RA than an individual who chain smoked (consecutively smoked one cigarette after another) cigarettes at a social gathering once during their youth. However, both duration and intensity do play a role in RA development. Interestingly, autoantibody production in smokers is much higher than nonsmoking individuals, additionally, even if smokers test negative for RF and ACPA, their risk for RA is still heightened compared to nonsmokers. CS breaks the tolerance the immune system has for autoantigens naturally present in the body, according to Ishikawa et al., thus triggering RA development. While both RF and ACPA are the most common autoantibodies present in people with RA, RF is present at higher rates within smokers than nonsmokers, while ACPA-positivity increased dramatically in the smoking population.

The effects of CS are of both short term and long-term detriment. There reaches a certain point that the damage from chronic CS in ACPA-positive patients can no longer be undone thus decreasing RA treatment outcomes. On the other hand, if a person has ceased smoking for more than twenty years, the level of ACPA-negativity increases along with improved outcomes for RA
treatment and potential remission. The potential improvement of RA outcome for smokers in longtime remission is an implausible outlook for chronic smokers. This stark contrast is once again thought to be due to the importance of duration verses intensity. Crucially, CS is not only detrimental when experienced first-hand, but also in a second-hand, “passive,” capacity. Children who experience passive CS have indicated a greater risk for RA in adulthood though this is a tentative correlation and must be further explored. This observation further solidifies the hypothesis that duration and intensity play key roles in how CS influences RA susceptibility.

*Cigarette Smoke Immune Impact*

Without question, CS impacts both cellular and humoral immunity, thus inducing a systemic inflammatory response both via the innate and adaptive immune systems. The difficult factor in CS is the way it impacts the human body is diverse and far-reaching. Direct inhalation of CS, as well as passive CS, is a known cause or strong factor in many diseases, including but certainly not limited to, heart disease, stroke, diabetes, lung disease, lung cancer, COPD, sudden infant death syndrome, and asthma. Therefore, to determine the exact mechanisms by which CS passive or active affects RA is no small feat (Figure 8).

One repercussion of CS is observed in Th cells acting with skewed effect in RA. Th17 in particular is a cell of interest in RA development due to its active role in signaling for neutrophils and inflammatory cytokines from other T cells during the early stages of RA. As divulged in the *Current Treatments* section, many pro-inflammatory cytokines proliferate exponentially during RA pathogenesis. Th17, TNF-α, IL-6, IL-1α/β, and IFN-γ all proliferate at staggering rates, and have therefore been targets for treating RA via DMARDs such as methotrexate or JAK inhibitors. CS becomes a catch-twenty-two when treatment options are analyzed however since at first glance it would seem as though smokers have many options for pharmaceutical
treatment due to the increased presence of target molecules for said therapies. CS adds yet another layer of complexity to treating RA however by negatively influencing the effectiveness of these drugs.\textsuperscript{6} Drugs such as infliximab, a biologic DMARD, therefore has little effect on the disease.\textsuperscript{6,24}

Figure 8: Pathogenesis of Rheumatoid Arthritis. Genetics and environmental factor interactions for rheumatoid arthritis. Results of this relationship leads to loss of tolerance of self-proteins such as B and T cells. Transitioning to arthritis this translates to structural damage of the joints, and other bodily systems.\textsuperscript{23}
Vaping and E-Vapor Components

Smoking e-cigarettes, commonly referred to by younger generations as “vaping,” continues to grow in popularity within the United States, and globally.\textsuperscript{25} Within the last year, a national study indicated that more than 10\% of youths ages 15-17, and 11\% of 18-21 year-old young adults have smoked Juuls.\textsuperscript{7} This does not include any of the other forms of e-cigarettes (electronic cigarettes) on the market, of which the CDC indicates there are many.\textsuperscript{7} Interestingly, vaping, unlike cigarette smoking, has a large appeal to broad groups of people, therefore, there is a more nonchalant acceptance of it amongst school-age children, as well as adults, regardless of their distinctly differing opinions concerning the harms of CS.\textsuperscript{26} In fact, many young people realize the harmful effects vaping has been shown to lead to, but the social aspects of vaping outweighs those facts.\textsuperscript{26}

Ranking from least to most popular for customer consumption, disposable e-cigarettes, rechargeable e-cigarettes such as Juuls, and tanks or mods are a few of the broad categories available to “vape” with (Figure 9).\textsuperscript{25} Juuls are particularly desirable in the eyes of middle and high schoolers because of their compact size and USB drive appearance which allows for quick and unassuming storage, however Juuls are a highly effective vessel for nicotine inhalation as well.\textsuperscript{25} The benefit to Juuls are that they seem to be less harmful than CS or other e-cigarettes in term so the carcinogens present within them.\textsuperscript{7}

\textit{Figure 9: Various forms of vape devices as described by the Center for Disease Control} \textsuperscript{25}
Within the vaping industry, each smoking mechanism product appeals to a different purpose of the user. For instance, disposable electronic cigarettes are the most affordable option and provide users affordable and speedy mechanism for nicotine.\textsuperscript{27} Traditional e-cigarettes are considered a weaker form of vaping.\textsuperscript{27} Box mods are incredibly popular due to their powerful, sub-ohm systems. Sub-ohm systems are often set to lower temperatures to allow the user to deeply inhale the e-vapor aerosol into his/her lungs. These models are more sophisticated than other vaping options, with complex systems to control various wattage settings, temperature control options, and are able to hold large amounts of e-liquid.\textsuperscript{27} These are often the models utilized by \textit{experienced vapers} because of their high capacity for release of aerosol, which allows for various tricks and smoke maneuvers to be performed.\textsuperscript{27}

All of the available e-cigarettes, hereby referred to as vaping devices, share common generalities in their functions. They produce an e-vapor aerosol (of which contains various chemicals, flavors or addictive substances) by heating an e-liquid, “juice,” via battery power producing heat via coils.\textsuperscript{25}

The general consensus on e-vapor aerosol inhalation, referred to as \textit{vaping} is that it is considered less detrimental than CS.\textsuperscript{28} This viewpoint stands as popular opinion since these modes of nicotine inhalation contain fewer carcinogens than cigarettes for those hoping to quit smoking cigarettes.\textsuperscript{25} There are also non-nicotine forms of juice available to be vaped depending on what type of model is used, and studies have indicated that these do not lead to compromised bone integrity.\textsuperscript{28} It seems as though even without the component of nicotine being introduced to an organism, RA is perpetuated due to the alternative chemicals and toxins within cigarette smoke.\textsuperscript{9} On the contrary, research also indicates vaping is likely as detrimental, if not more, than CS, depending on the model and juice utilized.\textsuperscript{7,25}
E-cigarette aerosols contain volatile organic compounds, nicotine, ultrafine particles which are detrimental to the lungs, heavy metals such as nickel, tin and lead, cancer-causing chemicals, and flavoring such as diacetyl (which is linked to serious lung disease).\(^{25}\) There are generally fewer carcinogens within e-cigarettes than traditional cigarettes (TC), however, they are replaced by propylene glycol (PG), vegetable glycerin (VG) and flavoring which masks the taste of toxins (unlike that of traditional cigarettes TC).\(^{29}\) Table 1 indicates the five most common ratios for these two liquids alongside the most commonly purchased vape juice flavors.\(^{27,29}\) Each concentration of vape elicits a different vape experience, thus juices are chosen based on individual preferences. Additionally, vape juices commonly contain anywhere from 0 mg, 3.0 mg, 6.0 mg, 12.0 mg, 18.0 mg, or 36 mg of nicotine per 1 mL which allows users to pick and choose their desired nicotine level for many of the juice options currently on the market.\(^{28}\)

\textit{E-Vapor and Adolescents}

\begin{table}
\centering
\begin{tabular}{|l|l|}
\hline
\textbf{Vape Juice Breakdown} & \\
70 PG & 30 VG \\
50 PG & 50 VG \\
30 PG & 70 VG \\
20 PG & 80 VG \\
N/A & Max VG \\
\hline
\end{tabular}
\end{table}

Table 1: Vape Juice Breakdown and Various Juice Flavors. VG is a thicker liquid thus eliciting a thicker more robust vapor with little flavoring. PG, however, is a thinner liquid ideal for flavor carrying and inhalation felt on the throat. Therefore, vape juice is often purchased and utilized depending on an individual's user's preferences.
Vaping is a fairly new form of smoking that is everchanging with its growing appeal due to its hypothetical superiority to CS.\textsuperscript{30} Though e-cigarettes are hypothesized to be a less harmful method for TC smokers to quit smoking or improve chronic asthma as well as COPD outcomes, there is a downside to e-vapor.\textsuperscript{30} Vaping has gained widespread popularity amongst adolescents and young adults. According to the American Journal of Preventative Medicine, youths view tobacco harm on a spectrum ranging based on the vesicle by which it is exposed to the body.\textsuperscript{31}

In 2017, electric cigarettes were the most regularly purchased from of tobacco by middle school and high school aged adolescents.\textsuperscript{30} An additional 1.3 million teens began vaping in 2018, alone. One can only speculate as to how many youths are partaking in vaping in the year 2020-approximately 16 million youths is once recent estimate.\textsuperscript{30} Unfortunately, vaping companies quickly realized the appeal vaping had amongst youths, and began marketing on social media platforms, receiving celebrity endorsements, and promoting vaping with cartoon images, sexual appeal and sleek marketing tactics.\textsuperscript{30} The perception of vaping being safe was therefore compounded by the advertising of these companies.\textsuperscript{30,32} These companies have since been rebuked and directed to refrain from such forms of advertising, however, the attraction to vaping lives on amongst middle and high schoolers.\textsuperscript{30}

\textit{E-vapor Effects on the Body}

Vaping has been shown to greatly affect oxidative stress (OS) due to an increased generation of reactive oxygen species.\textsuperscript{30} Reactive oxygen species play a detrimental role in health, often contributing to pathogenesis of diseases that occur in the respiratory system, metabolic actions and neurodegenerative diseases.\textsuperscript{30} Oxygen reactive species are also key players in the effects of addiction and dependence.\textsuperscript{30} These species play a role in smoking addiction, morphine addiction, alcohol and cocaine addiction and methamphetamine addiction.\textsuperscript{30}
Specifically, vaping could be an influential contributor to depression and suicide, sleep deprivation and attention deficits, as well as aggressive, impulsive behaviors. Ultimately, these outcomes are not on the list of characteristics commonly associated with the success for the future generation, therefore, it is imperative to study the true effects of vaping in order to better understand the long term effects it may have on adolescents. Though there are many contributors to the steady increase in depression and suicide within the United States, TC smoking is strongly associated with major depressive disorder among teens. Similarly, vaping use is associated with an increase in suicide ideation- as seen by an increase in reactive oxygen systems and their link to suicide and suicide attempts. Beyond mental health, adolescents who vape have been shown to express lower academic performances than their non-vaping peers. Cognitive deficits, memory impairment, attention deficits and alertness, and the disturbance of the development of the cerebral cortex and hippocampus are all strongly associated with TC smoking. At this point, it is critical to note that both first and second hand smoke from cigarettes has been shown to effect teenage sleep patterns, sleep deprivation, and therefore poor academic performance. There needs to be extensive further studies concerning the effects on adolescent health specifically pertaining to e-vapor, however. One study indicated the effects of vaping was not, in fact, from the vapor itself, but rather the effects of nicotine in the aerosol. Yet another study contradicts that data and indicates that oxygen reactive systems increased after e-vapor alone. Due to the vast options available for vaping juice flavors, nicotine or non-nicotine content, as well as the density of the vapor and aerosols, countless studies with various combinations could be performed. Lastly, long term studies must be performed to analyze the long term effects of vaping on teens and young adults; only minimal research has been performed in this area, pertaining to less than four years after vape exposure.
The general population often considers smoking TC or e-vapor with their potential effects on respiratory health. This association is well founded for TC, while the little research conducted on e-vapor effects is indicative of strong negative effects. One study discovered that the summer of 2019 held a tremendous upward trend of vaping-induced lung injuries (EVALI). In particular, vaping THC (tetrahydrocannabinol) correlates strongly with lung injuries; these correlations do not have bias impact based on age bracket. Whether young or mature adult, the effects of vaping THC specifically have proven to be detrimental. Young males who vape have been shown to experience fever, chills, headaches, chest pain, coughing and shortness of breath. Correspondingly, gastrointestinal issues such as pain, nausea and vomiting are common. The effects of e-vapor are characterizable even after two days of exposure; acute lung injury, diffuse alveolar damage and organizing pneumonia are a few of the quickly onset conditions perpetuated by vaping. In conjunction with these disease onsets, chest CAT scans indicated ground-glass opacities (Figure 10).
In comparison to TC, there is little data on the true physiological effects of e-vapor via the newest forms of e-cigarettes known as vape pens, mods or tanks. This lack of data leaves much to be desired in terms of the repercussions of vaping—particularly its effects on the next generation. With the increasing appeal to vaping due to unique smokable flavors, and diverse tricks and purposes behind each type of vaping, the adolescent population continues to consume this product with minimal evidence as to how it is impacting their health days, months and decades from now.

![Ground-Glass Opacification](image)

*Figure 10: Ground-glass Opacification. CAT scan reveals normal lung (right) versus ground-glass opacification (right). Ground-glass opacification within the lungs is common in patients with histories of chronic smoke inhalation, such as with vaping.*
Laboratory Research Proposal

Rheumatoid Arthritis is the leading form of arthritis, as well as one of the most prevalent autoimmune diseases. Likely initiated by gene expression abnormality, as well as environmental factors such as cigarette smoking, and potentially e-vapor, RA pathogenesis is complex and treatment plans complicated. Though this disease is most common amongst women who are forty years of age or older, RA susceptibility has been shown to increase with CS exposure. Since vaping was first introduced in 2007 it has become one of the most popularly consumed form of nicotine products on the market. In particular, vaping has a large appeal amongst adolescents. Cigarette smoking has been shown to increase the likelihood of RA induction exponentially, therefore there may be a similar connection between RA susceptibility and inhalation of e-vapor. Little research has focused on the effects e-vapor may have on the body; therefore, the need arises for this novel study. Primarily, studies as to whether or not vaping worsens the progression of RA must be explored; furthermore, the HLA-DRB1 gene expression pathway, as well as autoantibody (RF and ACPA) proliferation should be investigated since these factors are key players in CS exposure and RA pathogenesis.

Specific Aims

The objective of this research proposal is to determine the potential effects of vaping on adult susceptibility and the pathogenesis of rheumatoid arthritis. Moreover, the effects of chronic e-vape inhalation in adolescents and young adult will be analyzed to determine in order to determine a potential increase in rheumatoid arthritis onset later in life. These experiments will be carried out in arthritis induced mouse models.
Preparing Arthritic Mouse Models

All of the mice will be treated according to the appropriate handling of vertebrate animals as laid out by national guidelines and study protocol and kept within individually ventilated cages in 12-hour light/dark cycles. Likewise, food and water will be available to the animals ad libitum. They will also be acclimated to the nose-only exposure chamber prior to experimental vapor exposure. The background genome for both the experimental, sham and control groups of this experiment are C57Bl/6 mice. Historically, active immunization causing collagen induced arthritis most closely resembles that of RA within mouse models, so this will be induced in the mice. At 8-10 weeks of age the mice will be injected with a 100 ug tail base injection of CII dispersed in complete Freunds adjuvant (CFA) at day zero. This method was chosen because CFA allows for a water-in-oil emulsion of antigens that act upon the synovium of the joints. At day 21, these mice will receive a boost of another 100 ug CII of incomplete (IFA) to ensure proper induction of arthritis via plasma cell activation.

CRISPR-CAS9

Discovered in 1987 and further understood in 2007, CRISPR-Cas9 is a new technology based on a system naturally found within bacteria to ward off viral infections. Two short RNA chains form a complex with the Cas9 nuclease (DNA cutting) protein. This complex allows Cas9 to identify the portion of the viral DNA that is detrimental to the bacterium via a guide RNA. This specific portion of DNA is then cut from the genome and replaced with a target RNA strand. If the match is completed, then the DNA is cut, forcing the cell to begin the repair process. Due to errors that occur in the repair process, however, the gene which CRISPR interfered with becomes inactivated, allowing the observation of the function of a now disabled gene. This technology is applicable in any genome, even humans, and certainly mice, therefore,
it will be utilized within Specific Aim II in order to study the downstream effects of silencing HLA-DRB1.37

Specific Aim I: Vaping worsens Rheumatoid Arthritis.

This aim primarily seeks to lay the foundation for all following experimentations. Cigarette smoking has long been considered the strongest environmental factor to contribute to rheumatoid arthritis (RA) induction. The pathway of said influence has yet to be solidified, however, with the HLA-DB1 pathway presented as the most likely expression pathway for the immune changes that ultimately lead to onset of RA. Therefore, based on the most readily available research, this proposal hypothesized that e-vapor and its components may follow a similar pathway for RA induction as CS. Regardless of pathway however, it is proposed that vaping will induce more severe RA symptomology than non-vape exposed mice. There is a great need for this particular study due to the exponential rise of popularity for vaping amongst adolescents and young adults. The trend of vaping has only continued to grow since it was first introduced 2007, with little to no hard data and research on the repercussions of e-vapor.

30 C57Bl/6 CIA mice will be equally split between two groups of experimental mice. Additionally, the sham and control groups will consist of 10 mice each. The experimental and sham groups will be placed on an inhalation regimen for CS, e-vapor and filtered air, respectively via a nose-only aerosol exposure system Figure 11.28,34,38 The experimental mice will be exposed to nose-only inhalation for up to 4 hours/day, 5 days a week (a total of 14 days) in order to mimic chronic CS/e-vapor/filtered air inhalation.28,34,39 Conversely, the animals will be allotted time to acclimate to the nose-only inhalation and therefore exposure time will increase from 1 to 4 hours by one hour increments for the first four days of treatment.40 The mice
will be exposed to an average of one puff of smoke/vapor per 30 seconds, with a 3 second puff duration. The protocol for exposure is carried throughout Aims I-III.

Mice exposed to CS will inhale 3R4F Kentucky reference cigarettes, as these are commonly utilized within CS studies. E-vapor mice will be exposed to a 50PG/50VG/4% nicotine vapor based on weight consistent with a previous study concerning vapor effects. The mode of vape utilized will be via a SMOK RPM 80 POD MOD which is known to be the middle ground mod style for beginner and expert vaping individuals since the goal of this proposal is to, as accurately as possible, analyze the impact of vaping as it is performed by adolescent consumers in the future aims. The specific dimensions and features of this mod can be found in Supplemental Data 3.

![Figure 11: Nose-only Aerosol Exposure System. Utilized to standardize the inhalation and exposure time of mice to the various experimental smokes (CS, e-vapor, filtered air). Each mouse is housed within an individual tube with its nose exposed only to the central chamber which is pressure controlled to allow even distribution and concentration of the aerosols from attached generators.](image)
Several studies have been conducted concerning the effects of simplistic e-cigarettes, but little to no research has been performed in the area of the most common forms of vaping to date.

The central hypothesis for this experiment is that RA expression in both the CS and vaping group is affected, while it is not affected in the sham and control models. This will be determined by using a clinical arthritis score of a possible 28 points, paw volume, ankle thickness measured via caliper and ELISA to determine ACPA and RF autoantibody levels within blood serum and synovium.\textsuperscript{35,39,42} These results would indicate not only a solidification of numerous previous studies pertaining to the effects of CS, but also the potential correlation between vaping exposure and a perpetuation of RA in the CIA mouse models.

Specific Aim II: To determine whether RA is worsened when exposed to vaping in HLA-DRB1 knockout (KO) models. The C57B1/6 CIA mouse model, and a CRISPR-Cas9 modified mouse model will be used, respectively. Therefore, the groupings for this experiment will be as follows: 15 C57B1/6 CIA mice with nose-only exposure to vape, 15 C57B1/6 CIA, HLA-DRB1 KO mice exposed to e-vapor, 15 sham mice inhaling filtered air via nose-only exposure, and 10 control C57B1/6 mice.

The levels of autoantibody proliferation will be measured before and after treatment via ELISA, RA symptomology analysis will follow suite to Aim I protocol, and a micro assay will be performed to yield data on gene expression. In order to study the effects of HLA-DRB1 in experimental C57B1/6 mice with CIA, micro arrays will be utilized following the protocol previously stated.\textsuperscript{43}

The perspective outcomes for this aim are to see a significant increase in autoantibody presence within the synovium and blood serum of mice exposed to vapor. This promising result would advance the current hypotheses that autoantibodies are a necessary prerequisite to RA
pathogenesis. Addedly, one would anticipate the HLA-DRB1 manipulated upregulation to worsen RA, regardless of a vapor-less exposure. Though this particular focus of the study may prove unsuccessful in the desired results of HLA-DRB1 pathway upregulation triggering RA pathogenesis, the results would be of benefit since this would create opportunities to pursue alternative pathways for RA activation.

**Specific Aim III:** Lastly, the long-term effects of vaping on adolescent mice must be analyzed. There are currently no longitudinal studies concerning the effects of vapor on adolescent growth, development, and later the potential onset of RA. Due to the increasing popularity of vaping amongst middle school and high school aged students, this study is imperative to the health of the future generation. This long-term exposure study will be performed on adolescent CD57Bl/6 mice ranging from the juvenile age of 2 to 3 weeks of age continuing in observation until they reach 4 months of age.

The experimental groups will be separated based on the longevity with which they are exposed to vapor. The first set of 15 CD57Bl/6 mice will be exposed for 14 days. After which they will be analyzed for autoantibody production, as well as the same criteria for RA onset as in specific aim I. Another experimental group of 15 CD57Bl/6 mice will be exposed to nose-only aerosols for two months, with the final 15 mice experiencing inhalation of vape for the full 4 months to mimic chronic vaping tendencies. The control and sham groups remain the same as former experimentations have listed, except for their juvenile rather than adult state. Each of the experimental groups will be observed daily during the first two weeks of exposure, then then biweekly until they reach 6-8 months of age.

In the final analysis of these experimental groups, the proposed results would demonstrate not only an elicitation of RA, but also characteristic increases in its expression and
symptomology due to chronic inhalation of vaping aerosols within the juvenile mice. If such results are uncovered, this translates to a potentially severe health outcome for teens and young adults who chronically smoke using e-vapor mechanisms such as the SMOK RPM 80 mod utilized in this study. The exponential increase in vaping since it was first released in 2007 for public purchase could be setting up this next generation of young people for higher rates of RA susceptibility when they reach their mid to late adulthoods. Not only that, but perhaps this RA induction due to vaping will cause excruciating and escalated pathogenicity of the disease state., as CS has been hypothesized to do.

*Experimental Pitfalls and Future Aims*

These experiments are anticipated to shed light on the relationship between e-vapor and the induction of rheumatoid arthritis as it pertains to adult and juvenile mouse models. Be that as it may, there are number of pitfalls within this proposal. The first major pitfall is in the form of the vape analyzed within the study. Most notably, standardizing the amount of vapor each animal is exposed to proves difficult considering how unregulated the manufacturing sector for vape devices is. Though the SMOK RPM 80 POD MOD may be a sound middle ground between simplistic and complex vaping apparatuses, it is a small portion of the potential forms of vaping mechanisms on the market. Due to the vast array of options available for vaping ranging from PG and VG concentration levels, nicotine content, an endless supply of flavorings, and the apparatus utilized to create vape, there are multitudes of combinations that need to be explored regarding the effects of vapor. Furthermore, there is currently no data depicting the longitudinal effects of vaping (except for a single study which examined the effects of vaping three years after exposure). Though there are numerous studies on the broad topic of e-cigarette smoking, few have focused on vaping specifically- thus leaving much to be desired for the field.
Specifically focusing on the experimental design, ideally, more animal models would be utilized in order to study this subject matter in a more robust format. Moreover, there certainly should be a study which focuses on the expression of HLA-DRB1 as it relates to RA. The data on this genetic pathway indicate strong correlations between this and that of CS, so HLA-DRB1 in-depth investigation needed to be further analyzed both in CS exposure and vaping. In the future, it would prove beneficial to upregulate HLA-DRB1 expression without the presence of vape or CS in order to enhance results that support the role of this pathway in RA pathogenicity. Lastly, experiments which primarily focus on passive vaping, rather than direct inhalation of vape aerosols, would undoubtedly prove beneficial. Since vaping is more widely accepted as opposed to CS, individuals are able to vape in locations TC smokers are not permitted, thus exposing the general population to e-vapor. In short, the list of future experimentations concerning the effects of vaping appears to be ever-growing as the industry continues to grow in popularity, particularly amongst younger generations.
Conclusion and Future Aims

Rheumatoid arthritis is the leading form of arthritis globally, and commonly begins its effects on individuals in their mid to late 40’s. Nearly two percent of the global population is affected by the degrading symptoms of rheumatoid arthritis including, but not limited to, swan neck and boutonniere joint deformation, pain, inflammation, degreased mobility and decreased quality of life. There is an array of current treatment options for RA that offer various tactics to combat the pains of RA, but with short-term and complex treatment plans a cure has yet to be discovered.

RA is most often characterized by the presence of autoantibodies in the pre-onset stage of disease progression ACPA and RF being of highest significance for disease pathogenicity. Though these two autoantibodies cannot be utilized as the only formal indicators of RA induction, they are the most common signs for RA onset and are observed in pre-clinical stages of the disease. Additional indicators such as an increase in fibrinogen synthesis, an increased presence of TNF-α and IL-6, as well as Th17 cells produced cytokines: IL-17A, IL-17F and IL-22 during active RA. The notable complexity of RA begins with the conundrum of what the initial step of RA truly is. There is large “chicken or the egg first issue” in terms of whether autoantibodies or self-reactive T-cells and their related cytokines are the initial perpetrators to RA induction. However, there is large scientific consensus considering the genetic and environmental factors which contribute to an increased susceptibility to RA.

Firstly, the HLA-DRB1 pathway is thought to be the primary pathway for genetic expression which leads to the increased proliferation of autoantibodies such as ACPA and RF. Secondly, the environmental factor of cigarette smoking is the most widely accepted external contributor to an increased propensity towards developing RA, regardless of gender.
particular is hypothesized to be the trigger which causes the immune system to lose its self-tolerance and thus lead to an autoimmune attack against itself. The exact mechanism for how this occurs is still uncertain, however. Additionally, the HLA-DRB1 gene and its related pathway is the most noteworthy genetic potential for RA influence; it is not the exclusive pathway for how RA may arise from a genetic perspective. Due to the many factors which perhaps lead to an increased vulnerability to the inclination of RA development, the need arises for further determination of the mechanisms behind rheumatoid arthritis pathogenicity. Given the many questions concerning how an individual may be predisposed or superimposed to RA due to environmental agents, as time continues, new environmental factors may play a role in RA. Therefore, the recently introduced form of smoking known as e-vapor, “vaping,” may be yet another powerful contributor of future RA generation.

Vaping is most commonly utilized by adolescents and young adults. Since the data on the longitudinal effects of vaping has yet to be collected beyond a three-year time period, there is an imperative need for the long-term detriment vaping may cause- including potentially leading to RA, as seen with chronic cigarette smoking. Therefore, the experimental proposal within this thesis focused on not only the potential connection vaping has with triggering RA, but also chronic vape exposure and how it effects RA severity, in respect to both the HLA-DRB1 pathway and HLA-DRB1 knockout mice. There are heaps of future experiments which could be performed specifically focusing on the numerous forms of vaping, but first-and-foremost there must simply be any form of these experiments performed. Second-hand vaping, analysis of alternative pathways for RA expression, and comparisons between the effects of vaping on adults versus children are but a few to consider. Experiments on vaping must be performed due to the drastic increase in popularity it has amongst youths. In 2018, 1.3 million middle and high school
students were vaping, however, just a mere two years later, over 16 million adolescents within the United States have tried or consistently vape.\textsuperscript{7,25} Consequently, the future generation perceives vaping as the healthier alternative to cigarette smoking, but the effects of vaping on bodily health is largely unknown. Id est, though vaping is seen in a fairly positive light by many, it may be as deleterious as CS in terms of its short and long-term health consequences.

Upon final analysis, rheumatoid arthritis is one of the most common autoimmune diseases within the United States, and globally. Though there are many potential contributors to an increased susceptibility for inducing RA, two main factors must be highlighted: the effects of one’s genetic predisposition, and the environmental factor of cigarette smoke. With the vaping industry only continuing to gain appeal amongst adolescents, the question is raised as to if, and how e-vapor potentially contributes to the onset of rheumatoid arthritis as these individuals reach late adulthood. Without studies which focus on this potential connection to RA pathogenicity, there may be a population which is voluntarily increasing their vulnerability to rheumatoid arthritis.
References


35. Antibodies Specific for Carbamylated Proteins Precede the Onset of Clinical Symptoms in Mice with Collagen Induced Arthritis.


Supplemental Data

Supplemental Data 1: Example of a microarray. This particular example of a microarray depicts the comparison of osteoarthritis (OA) and rheumatoid arthritis (RA). The original caption is included to provide context for the significance of this example. Note the RA specific genes (highlighted in yellow) in both a and b. Experimentation similar to this was performed in the “Experimental Proposal” portion of this paper.

Figure 2. Expanded view of distinct gene expression signatures of selected differentially expressed genes between OA and RA samples. Expression level was normalized per gene, data were log-transformed and the relative value to the mean among the 15 samples is shown by color: red; relatively high expression, green; low expression. The clustering program arranges samples (10 OA and five RA) along the horizontal axis so that those with the most similar expression profiles are placed adjacent to each other. The organization of the results was made with the same number of genes and arrays used in the experiment and means of redundant hits were used. Rows represent genes (unique cDNA element) and columns represent experimental samples. OA = osteoarthritis, RA = rheumatoid arthritis. (a) Expanded view of distinct gene expression signatures of 63 selected differentially expressed genes between OA and RA samples. (b) Expanded view of distinct gene expression signatures of 48 known selected differentially expressed genes between OA and RA samples.
Supplemental Data 2: Established by the European League Against Rheumatism (EULAR), this is the recommended pathway to treatment options for rheumatoid arthritis (RA) and its management. Broken into three phases, pharmaceutical options include csDMARDs, bDMARD, DMARD, biologic agents, and JAK-inhibitors.22
**Supplemental Data 3: Mode of Vape Utilized for Experimental Proposal.** SMOK RPM80 is considered a median level vape device due to its 0.4-ohm system, customizable temperature gauge and display screen. Additionally, this model is not as industrial as other models due to its moderate puff capacity.

**SMOK RPM80 Pod Mod Kit Features:**
- IQ-80 Chipset
- Dimensions - 109mm by 31.55mm by 26mm
- Integrated 3000mAh Rechargeable Battery
- Wattage Output Range: 1-80W
- Voltage Output Range: 0.5-4.0V
- Resistance Range: 0.15-3.0ohm
- Zinc-Alloy Chassis Construction
- Intuitive Firing Button
- 0.96" OLED Display Screen
- Two Adjustment Buttons
- SMOK RPM and RGC Pod & Coil Series
- Side Refill System - Silicone Stoppered
- 5mL Refillable RPM80 Pod
- 0.4ohm RPM Mesh Coil
- 5mL RPM80 RGC Pod
- 0.17ohm RGC Conical Mesh Coil - rated for 0.5-1.0ohm RGC RBA Coil - Coming Soon
- Plug 'n' Play Coil installation
- Adjustable Airflow Control
- Magnetic Pod Connection
- Low Battery Protection
- Short-Circuit Protection
- 10S Cut-Off Protection
- Over-heating Protection
- Intelligent Atomizer Recognition
- Puff Monitoring System
- Bottom MicroUSB Type-C Port
- Available in Black Stabwood, Red Stabwood, Fluid Gold, Fluid Blue, Black and White Resin, 7 Color, and Black Carbon Fiber