

University of Nevada, Reno

**Asthma: Cytokine-Induced Over-Expression of Matrix Metalloproteases
Compromises Airway Epithelium Tight Junctions and a Clinical Case Study**

A thesis submitted in partial fulfillment
of the requirements for the degree of

Bachelor of Science in Neuroscience and the Honors Program

by

Erika Cordero Mauban

Jonathan Baker, Ph.D., Thesis Advisor

May, 2016

**UNIVERSITY
OF NEVADA
RENO**

THE HONORS PROGRAM

We recommend that the thesis
prepared under our supervision by

ERIKA CORDERO MAUBAN

entitled

**Asthma: Cytokine-Induced Over-Expression of Matrix Metalloproteases
Compromises Airway Epithelium Tight Junctions and a Clinical Case Study**

be accepted in partial fulfillment of the
requirements for the degree of

BACHELOR OF SCIENCE, NEUROSCIENCE

Josh Baker, Ph.D., Thesis Advisor

Tamara Valentine, Ph.D., Director, Honors Program

May, 2016

Abstract

Asthma is a chronic respiratory disease that results in a constriction of the airways. While there is no known cause of the disease, one possible theory of the molecular cause of asthma is that the tight junctions of the airway epithelium are disrupted as a result of cytokine activity that up regulates expression of matrix metalloprotease 9. MMP9 encourages detachment-induced cell death—anoikis—which results in a structural change compromising the airway epithelium’s ability to act as a primary immune defense mechanism. The chronic characterization of asthma results because of an ineffective epithelial repair mechanism which prevents full recovery of the affected airway tissues thus allowing for further aggravation by environmental pathogens. Further investigation of this pathway could potentially allow for improvement in treatment and perhaps even the identification of a cure. First, a detailed background of the disease will be introduced in chapter 1. The following chapter will provide a literature review focused on the mechanism by which matrix metalloprotease 9 results in a compromised airway epithelium (and thus increased susceptibility to environmental pathogens). Lastly, chapter 3 will comprise of a clinical case study regarding the diagnosis and treatment of asthma.

Acknowledgements

I would like to thank Dr. Josh Baker for serving as my thesis advisor and helping me navigate through this thesis. You truly kept me motivated to work through this project. I would like to thank Megan Almansoori for keeping me on track and providing me with invaluable resources to facilitate the development of my clinical case study. I would also like to thank my mentor, Stephen Owens, MSIV, for his help developing my clinical case study. I appreciate the time and energy you dedicated to meeting with me in order to help me form my case study as well as to clarify my questions. I felt that you were truly invested in my understanding of asthma as well as my success on this project. Lastly, I would like to thank my family and friends for always supporting my academic ventures.

Table of Contents

Chapter 1	1
Abstract	1
Introduction	1
Clinical Features	1
Pathogenesis	1
Symptoms	2
Epidemiology	3
Genetics	5
Environmental Factors	7
Diagnosis	8
Treatment	11
Body of Review	13
Conclusion	13
Chapter 2	15
Abstract	15
Introduction	15
Methods	18
Results	20
Discussion	25
Chapter 3	30

Patient Introduction.....	30
Chief Complaint.....	30
History of Present Illness.....	30
Review of Systems.....	32
Social and Family History.....	32
Vital Signs.....	33
Physical Exam	34
Diagnostic Work.....	35
Differential Diagnosis.....	36
Diagnosis and Supporting Argument.....	38
Treatment Plan.....	40
Prognosis.....	43
Plan Implementation.....	44
References	46

List of Figures

Figure 1. Comparison of distribution and abundance of tight junction proteins ZO-1 and occludin among healthy individuals and patients of varying degrees of asthma.....	21
Figure 2. Increased permeability of airway epithelial tissue due to cytokine activity.....	22
Figure 3. GFP labeled basolateral membranes of untreated, EGTA, and MMP9 treated human epithelial cultures.....	23
Figure 4. Comparison of distribution of claudin-1 and occludin tight junction proteins between untreated and MMP9 treated tissue samples as well as comparison of cells present within continuous sample and those detached from sample.....	24
Figure 5. Comparison of growth between control and MMP9 treated samples over a 30 hour time period.....	25

CHAPTER 1

Review of Asthma

ABSTRACT

Asthma is a respiratory condition characterized by a constriction of the airways. Underlying pathogenesis as well as genetics and environmental factors predisposing individuals to the disease are studied. Clinical features, symptoms, diagnosis, and current treatment are reviewed. Global implications and future directions are assessed.

INTRODUCTION

Clinical features

Affected patients generally present with abnormal lung sound and respiratory inflammation. Observations consistent with asthma also including wheezing, swollen upper respiratory passages, and skin rashes thought to be caused by allergens (NIH 2014). Further indication of asthma can be determined during collection of medical history. The physician will collect information pertaining to frequency and intensity of asthmatic symptoms (such as wheezing and shortness of breath) as well as if any allergens or events cause these symptoms to occur or worsen. If a diagnosis of asthma is supported by medical history and the physical exam, lung function tests are likely to follow (see diagnosis).

Pathogenesis

While the exact cause of asthma is unknown, much research has allowed for the understanding of some basic molecular underpinnings of the disease. The pathogenesis of

asthma stems from many different levels of the airway which may act individually or in conjunction with another level. Possibly the most notable level of pathophysiology is the airway epithelium. In healthy individuals, this epithelial layer plays a role as a primary defense by trapping allergens in mucus, and using their cilia to move mucus and trapped allergens out of the airway. In order to fulfill this task, the epithelial layer must maintain tight junctions. However, in individuals with asthma, these tight junctions are disrupted, allowing pathogens to enter into the body and become sensitized. Such disruption results in development of allergies to the penetrating allergens which thus elicit an inflammatory response. Specifically, eosinophil recruitment results in the release of cytokines (a type of cell signaling protein involved in immune response) which contribute to the airway inflammation characteristic of asthma (Kudo et al., 2013).

Symptoms

Symptoms of asthma are highly variable in degree of severity and between patients. The most common symptoms of asthma include shortness of breath, breathing characterized by a slight whistle sound (wheezing), coughing that tends to exacerbate at bed down and bed rise, and tightness of the chest (NIH, 2014). If these symptoms are further aggravated, they can result in difficulty sleeping. More extreme symptoms are portrayed during asthma attacks which are specifically defined as an increased severity of swelling and inflammation of the air passages. These events generally result as a response to respiratory viruses, environmental allergens (such as pollution or pollen), and physical activity (Mayo Clinic, 2014). Treatment of such events can range from inhaler usage to

emergency care. Overall progressive worsening of asthma is noted by increased incidence of asthma attacks and intensity of symptoms (Mayo Clinic, 2014).

Epidemiology

Asthma, a significant contributor to global morbidity and mortality, accounts for 1% of the global disease burden, and is thus one of the most impacting chronic illnesses in the present world. In fact, it has been estimated that asthma results in the loss of approximately 15 million disability-adjusted life years each year. While it is understood that asthma affects a significant portion of the world, it is difficult to calculate exact frequencies of incidence since the definition of asthma is relatively subjective compared to other disorders. The inconsistency in such figures is probably due to the wide range of variable expressivity as well as the vast difference in frequency of symptoms between patients. Moreover, cases of asthma can be further categorized by age of onset or events that increase frequency of asthma attacks (e.g. pre-adolescent versus late onset and exercise-induced versus obesity-related) (Bernstein, 2014).

While exact calculations may be inconsistent, average estimates have illustrated that asthma is responsible for approximately 1 out of every 250 deaths worldwide. While asthma affects individuals of all ages and socioeconomic levels and is present in populations of all countries, the prevalence of asthma is not evenly distributed. Notably, asthma appears to be more common among the lower classes of high income, westernized countries. For instance, the cost of treatment for asthma in the United States in one year alone is approximately \$56 billion. Other English-speaking countries such as the United

Kingdom and Australia exhibit a much higher prevalence (as high as 36.8%) when compared to Mediterranean and Eastern European countries such as Greece and Uzbekistan (as low as 1.6%) (Bernstein, 2014).

Some racial disparities exist within the United States. For instance, African Americans and South Asian Americans exhibit higher frequencies of asthma. Since frequencies of these subpopulations within the United States do not correlate with frequencies of their native countries, it is highly unlikely that the racial disparities are due to genetics. Instead, it has been proposed that African Americans and South Asian Americans have a higher incidence of asthma for socioeconomic reasons. For instance, these groups tend to be low income, reducing the probability that they have proper access to consistent asthma treatment, many times forcing them to resort to emergency care. Further, low income populations tend to view this condition as insignificant when compared to other daily struggles such as securing food and shelter (Bernstein, 2014).

Asthma also exhibits different frequencies between the sexes at different stages of life. For instance, pre-adolescent asthma affects more males than females. In fact, for the age range of 0-10, males were hospitalized two times as frequently as females. This early onset form of asthma is the most common chronic childhood disorder. Other than implications involving responsibilities of children to medicate, asthma has significant, wide-ranging effects in that asthma-related hospitalizations also strongly affect attendance of primary school. Particularly, complications of pre-adolescent asthma peak around the age of 5 (Bernstein, 2014).

However, inclusive of all ages of onset, asthma affects more females than males. Particularly, for the age range of about 30-60, females were hospitalized about two times as frequently as males. Since the reversal of prevalence between the sexes change around the time of puberty, these frequencies may be explained by sex hormones. However, additional theories have been postulated concerning the relatively smaller size of female airway compared to that of males. (Schatz et al., 2003)

The rate of asthma diagnosis has increased in recent times. Specifically, there is a twofold increased risk associated with those born after 1966. In the United States, those hospitalized due to asthma attacks increased by 1.4% from 1980 to 1999. At the same time, U.S. asthma related mortality also experienced an increase (of 3.4%) (Bernstein, 2014). At the current rate of incidence, it is estimated that 100 million more individuals will be affected by asthma by the year 2025.

Genetics

According to one comprehensive 2006 study, over 120 genes have been recognized in peer reviewed journals to have a correlative association with either the susceptibility of asthma or its related symptoms (Bosse et al., 2007). These 120 identified genes can then be organized into distinct subcategories based on their associations within the asthmatic pathway: innate immunity, helper T cell differentiation, epithelial and mucosal immunity, and lung and airway function (Vercelli, 2008). These 120 genes were mostly identified using either candidate gene strategy or positional cloning approaches (Bosse et al., 2007). While candidate gene strategy requires prior knowledge of gene

function, positional cloning approaches are essentially a genome-wide scan that compares the genotypes of those affected with asthma and those individuals who are unaffected, and thus does not necessitate previous knowledge of genes.

Most of those genes identified had protein products that either function within the plasma membrane or are secreted and involved in the inflammatory response (Bosse et al., 2007). Three of the perhaps most commonly known and strongly supported genes involved in asthma are the vitamin D receptor, G-protein receptor A (GPRA), and ADAM33 (Bosse et al., 2007). In fact, ADAM33 was one of the first genes to be identified as having association with asthma. The vitamin D receptor is highly multifaceted in that it regulates the functions of many genes, especially those involved in the immune system. Much evidence has been provided to support that the vitamin D receptor plays a key role in asthma. In fact, both mice and humans who lack the vitamin D receptor gene are unable to participate in an inflammatory response at all (Bosse et al., 2007). Thus, they are completely resistant to asthma. The GPRA gene is also involved in the susceptibility for asthma. In both mice and humans who exhibit higher than normal levels of GPRA, the susceptibility for asthma is increased (Bosse et al., 2007).

Unfortunately, searches for genes involved in asthma are extremely challenging. This is due to not only the inconsistency in the diagnosis of asthma (see “Epidemiology” and “Diagnosis”), but also because the pathophysiology of asthma is so complex. Because asthma can stem from different pathways, the genetic causes of asthma are also highly variable. This idea becomes even more complicated when the possible effects of

gene interaction are applied (Bosse et al., 2007). Also, many gene regions that have been shown to associate with asthma may actually be composed of more than one loci, resulting in an additive effect that increases susceptibility (Vercelli, 2008).

On a more positive note, research searching for genetic links to asthma has not been in vain—it has led to further comprehension of the disease and has even brought about new theories about asthma’s pathology (Vercelli, 2008).

Environmental Factors

In general, an increase of incidence of asthma is strongly correlated with an urban, westernized lifestyle. Such environmental factors include diet, exposure to infection early on in life, household heating systems, and exercise. As discussed in “epidemiology,” incidence is much higher in English speaking countries in comparison to Mediterranean countries. One of the main reasons for this pattern is in diet. In terms of lipids in diet, Westernized diets typically include much more omega-6 fats in comparison to the Mediterranean diet which is more central on omega-3 fats (Bernstein, 2014). Omega-6 fats are polyunsaturated fatty acids that are typically found in foods such as vegetable oils and margarine. They promote asthma due to their tendency to increase the likelihood of inflammatory responses within the body. On the other hand, omega-3 fats which are commonly found in oily fish, have the opposite effect on the inflammatory mechanism. They are said to be anti-inflammatories, supporting the idea that they fend off asthma to an extent. Further, Mediterranean diet contains high levels of zinc and the

fat soluble vitamins A, D, and E, all of which are shown to prevent asthma (Bernstein, 2014).

Further, the increase in asthma incidence in more current times could be due to urbanization and the subsequent advances in technologies. For instance, in the past, most household heating systems were dependent on burning coal or wood. However, in modern times, household heating systems have shifted to energy sources such as natural gas and electricity. With this major revolution in energy sources, there has also been an associated increase in asthma incidence (Peden, 2000). Other technologies and luxuries that have come with urbanization, such as the automobile, have also contributed to asthma's increasing prevalence (Peden, 2000).

Other outdoor pollutants that increase the risk of asthma include sulfur dioxide, nitrogen dioxide, and ozone (Diette et al., 2008). While these outdoor environmental pollutants may quickly come to mind, indoor pollutants are also argued to be extremely influential on the development of asthma. For instance, indoor pollutants, such as molds and pet dander, as well as organisms which may infect the home, such as cockroaches and rodents, highly contribute to an allergenic environment, especially harmful to those already predisposed to asthma (Diette et al., 2008).

Diagnosis

Diagnosis of asthma is determined after taking medical history, carrying out a physical exam, and, often times, complete lung function tests. Specific medical history questions concerning symptoms and triggers are likely to be asked. For instance, analysis

of medical history will likely include what types of symptoms present, how often they present, and how intense they are as well as what (if any) allergens or events cause these symptoms to occur or worsen. During a physical exam, concerns for asthma can be further supported by listening to lung sound and looking for respiratory inflammation. Observations consistent with asthma including wheezing, swollen upper respiratory passages, and skin rashes thought to be caused by allergens (NIH, 2014). If a diagnosis of asthma is supported by medical history and the physical exam, lung function tests are likely to follow.

Lung function tests vary for patients of different ages. Specifically, spirometry tests (which are the most popular way to diagnose asthma) are more difficult to perform on children, with difficulty increasing as age decreases. In fact, pre-adolescent asthma is harder to diagnose in general because apparent wheezing symptoms could be due simply to the small size of airways consistent with young children. In such cases, wheezing generally dissipates on its own as the child (and thus his or her airway) grows. If the concern for asthma persists, spirometry tests are used. According to a New Delhi study, the most sensitive and thus successful measure of asthma in adolescents are forced expiratory flow values corresponding to the average flow rate between 25% and 75% of forced vital capacity (FEF_{25%}-FEF_{75%}) (Ratageri, 2001). Observed values are indicative of asthma if the value falls below approximately 60% of predicted value (Johns et al., 2008). However, if there are difficulties using spirometry with younger children, sometimes trial medications are given for a short but significant time frame, and any

improvements (or lack there of) are observed to determine if the child is affected with asthma.

In contrast, spirometry is a very common test used to diagnose adults. Spirometry can be used in conjunction with certain chemicals, where values are recorded before and after exposure. For instance, in a methacholine challenge test (MCT), methacholine, a drug that induces spasms of air passage muscles, can be administered for inhalation. The changes in values before and after treatment are thus compared to determine if the results are consistent with asthma (Khalid et al., 2009). However, instead of relying on FEF25%-FEF75% values, forced expiratory volume in 1 second (FEV1 values) is generally a better index for diagnosis in adults (Khalid et al., 2009). Asthma is diagnosed by a lower limit of around 70%; however, this limit is variable dependent upon age (Johns et al., 2008).

Once asthma is diagnosed, cases can be classified as either mild intermittent, mild persistent, moderate persistent, or severe persistent. Mild intermittent is characterized by no more than two days a week of daytime symptoms and no more than two nights a month of nighttime symptoms. Mild persistent patients exhibit more than two days a week but less than once daily of daytime symptoms and more than two nights a month of nighttime symptoms. Moderate persistent cases are distinguished by daily daytime symptoms and nighttime symptoms occurring more than once per week. Severe persistent is characterized by continual daytime symptoms and frequent nighttime symptoms. (NIH, 2002) In addition to diagnosing asthma, allergy tests may also be conducted in order to

comprehend what allergens exacerbate the condition. Such allergens are generally variable between individuals.

Treatment

A cure has yet to be developed. Instead, clinical goals are focused on controlling the symptoms and preventing their intensification. Some of the primary treatments prescribed are inhaled corticosteroids, long-acting beta-agonists, leukotriene modifiers, and theophylline (NIH, 2012). Treatment is dependent upon the degree of severity of the disorder, where the National Institutes of Health suggest that mild intermittent cases do not require daily medication, mild persistent patients should use a low dose of inhaled corticosteroid, moderate persistent individuals should use either low dose inhaled corticosteroids with long-acting beta-agonists or a medium-dose inhaled corticosteroid, and severe persistent patients should use a high dose inhaled corticosteroid in conjunction with long-acting beta-agonists (NIH, 2012).

Inhaled corticosteroids are the primary treatment of asthma; however, they may be aided by additional medications. Inhaled corticosteroids alleviate asthma symptoms by reducing the inflammation of airways. At a molecular level, they function within the nucleus, interacting directly with DNA to result in both an increased production of proteins that decrease inflammation and a decreased production of proteins that increase inflammation (Kercsmar et al., 2008). There are currently six types of inhaled corticosteroids on the market for clinical use. Their differences in pharmacokinetics allow for a variety of options to fit individual patients' needs.

Supplementing these inhaled corticosteroids with long-lasting beta-agonists can further alleviate asthma symptoms. Particularly, asthma attacks during the nighttime are highly correlated with hospitalization and mortality. Thus, another major clinical goal is to take preventative action against nighttime symptoms. While long-lasting beta-agonists are often prescribed for daily usage with inhaled corticosteroids (if used alone they can increase mortality), they are also highly effective in reducing nighttime symptoms (Mysore et al., 2011). In general, they are effective in reducing the risk of exacerbation of symptoms. They are, in turn, made more potent by inhaled corticosteroids which increase production of adrenergic receptors that present on the smooth muscles of the airways (Kercsmar et al., 2008).

Another medication used to prevent nighttime symptoms is theophylline—a bronchodilator taken orally. This treatment's effectiveness is dependent upon the fact lung capacity contains high variability based on circadian rhythms (Yassin et al., 2012). However, this medication is prescribed less frequently as users exhibit more side effects compared to more common treatments such as inhaled corticosteroids and long-lasting beta-agonists.

Another treatment that can be prescribed in conjunction with inhaled corticosteroids are leukotriene modifiers. Since many manifestations of asthma, such as increased mucus secretions and smooth muscle contraction, are the result of stimulation of the cysteinyl leukotriene (CysLT) 1 receptor, one treatment is preventing the stimulation of this receptor (Montuschi et al., 2010). Thus, CysLT antagonists can further

alleviate asthma symptoms. They are also shown to increase the effectiveness of inhaled glucocorticoids, allowing their dosage to be decreased (Montuschi et al., 2010).

BODY OF REVIEW

Asthma is a respiratory condition that manifests as a constriction of the airways. More specifically, a patient with asthma presents with abnormal lung sound and respiratory inflammation and expresses symptoms of wheezing and shortness of breath that may be exacerbated by environmental pathogens or exercise. Asthmatic patients are officially diagnosed via spirometry tests and are generally treated with inhaled corticosteroids which may be supplemented with long-lasting beta-agonists.

Environmental factors that exacerbate the condition include a diet high in omega-6 fats and industrial pollution. While many genes are said to increase risk of asthma, no specific genes of large affect have been found. However, genes involved in the production and maintenance of tight junctions in airway epithelial tissue is the main focus of current research.

CONCLUSION

Asthma is an increasingly prevalent disease, summing up to about 1% of the global disease burden (Bernstein, 2014). While the severity of the disease is highly variable between individuals, it has been estimated to result in the loss of approximately 15 million disability-adjusted life years each year, indicating a significant impact on quality of life (Bernstein, 2014).

A disease that is so widespread demands attention and further research. While there are many ideas surrounding the complex interaction of genes and environmental factors which predispose individuals to asthma, comprehension of the direct causes of asthma will enable researchers to develop a cure as opposed to a treatment aimed to minimize uncomfortable symptoms.

Future studies on asthma could likely focus on further developing a clear definition of the disease (in order to make diagnosis more objective and accurate) and a comprehensive mechanism underlying the disease, which combines the pathogenesis of asthma at the various different tissue levels and respiratory passages.

CHAPTER 2

Cytokine-Induced Over-Expression of Matrix Metalloproteases Compromises Airway Epithelium Tight Junctions

ABSTRACT

Tight junction disruption has been the primary suspect of many inflammatory diseases including inflammatory bowel disease, COPD, and asthma. Relative to healthy control patients, asthmatic patients exhibited decreased numbers of tight junction proteins, such as zona occludens and E-cadherins, along their respiratory epithelium as well as insufficient means of repair of the airway epithelium. Researchers have proposed that these depressed numbers of proteins are the result of excess cytokine activity. Particularly, pro-inflammatory cytokines increase expression of a class of matrix metalloproteases (MMPs). The hyperactivity of these proteases results in altered distribution and efficiency of tight junctions. Airway epithelium serves as one of the body's primary defense mechanisms against antigens. Thus, if the production, maintenance, and/or repair of tight junctions are compromised, as in the case of asthma, these individuals are more susceptible to infection, resulting in the observed clinical features.

INTRODUCTION

While asthma has been partially explained by many varying theories of pathogenesis occurring at many different tissue and respiratory tract levels, much research is still going into either finding a pathway common amongst all individuals affected with asthma—a comprehensive mechanism—or determining if asthmatic

conditions can be subdivided into more specific diseases depending on the varying degrees of severity as well as their respective pathways. However, as of present research, it is understood that each mechanism proposed for asthma does involve a complex interaction between both genetic predispositions and environmental risk factors. Specifically, one of the most strongly championed and substantiated theories is that a genetic predisposition to ineffective or weakened tight junctions, specifically those organized at the apical region of the airway epithelium, contribute to the increased susceptibility to and sensitivity of environmental risks factors (including pathogens such as pollution, bacteria, and viruses) which in turn result in the typical asthmatic inflammatory responses responsible for asthma patients' symptoms and clinical features.

The disruption of tight junctions in bronchial epithelium is a phenotype extremely common in asthmatic patients. This disruption has been traced back to a genetic component which involves the production of malfunctioning junction proteins such as zonula occludens-1 (ZO-1) and epithelial cadherin (E-cadherin), as well as an environmental component (Xiao et al., 2011). ZO-1 is a protein necessary for construction and stabilization of tight junctions. Specifically, ZO-1 functions as a scaffold for the production of tight junctions; its variety of domains allows for a number of differing junction proteins to bind to it (Nusrat et al., 2006). E-cadherin is a transmembrane cell-cell adhesion protein that is also imperative to the integrity of tight junctions in airway epithelium. The environmental component of tight junction disruption has been observed in mice which exhibit disrupted tight junctions when exposed to dust

and mites (Xiao et al., 2011). Thus, a combination of diminished or impaired tight junction proteins and exposure to dust and mites both contribute to compromised tight junctions in the airway epithelium.

In turn, the source of these malformed or absent junction proteins appears to originate from pro-inflammatory cytokine activity. Individuals affected with asthma exhibit allergic sensitization to common environmental allergens resulting in a cytokine response (Holgate, 2008). More specifically, this hyperactivity of the cytokines increases the expression of a particular class of matrix metalloproteases (MMPs). Normally, these proteases are produced by a variety of cells, including epithelial cells, and are responsible for degrading some constituents of the extracellular matrix (Vermeer et al., 2009). As the proper maintenance of the extracellular matrix is dependent upon proper functioning of this family of proteases, their production and activity are highly regulated. In fact, their transcription and activation (via cleavage of the zymogen) are regulated by cytokines and other proteases, respectively (Vermeer et al., 2009).

However, asthmatic patients exhibit not only an increased concentration of MMP9 in their airways, but also an altered concentration of tissue inhibitors for these metalloproteases (TIMPs). This imbalance suggests an alteration of MMP9 regulation in asthmatic individuals. The role of MMP9 in regards to the development of asthma was also supported by experiments with MMP9 knockout mice. These mice exhibited reduced arthritis and encephalomyelitis which suggests that MMP9 may function in inflammation. In order to test the theory that MMP9 encourages the epithelial damage responsible for

the manifestation of asthma in humans, researchers utilized human airway epithelium to evaluate MMP9 activity with respect to its effects on tight junction formation and function (Vermeer et al., 2009).

METHODS

In order to determine the significant difference in tight junctions between healthy individuals and asthmatic individuals, first bronchoscopy was used to collect bronchial epithelial samples from non-smoking individuals who had not experienced respiratory infection for the previous 4 weeks. Samples were then divided into two groups: healthy control patients and asthmatic patients (Xiao et al., 2011). Cultures were immunostained using anti-ZO-1 and anti-occludin antibodies then fixed. As standard, in order to increase the contrast for electron microscopy, the samples were then stained twice—once with uranyl acetate—a stain that functions by interacting with lipids and proteins—and then again by Reynolds lead—which involves a reaction between sodium citrate and lead nitrate in order to produce a lead citrate which directly stains the samples (Xiao et al., 2011; Leica Microsystems 2013). The stained samples were then analyzed using transmission electron microscopy (Xiao et al., 2011).

To investigate what causes this disruption of tight junctions, researchers completed in vitro experiments involving the addition of a variety of interleukins—including pro-inflammatory cytokines—to otherwise healthy endothelial tissue (Capaldo et al., 2008). Specifically, researchers collected healthy human bronchial epithelial cells, treating some with cytokines and leaving others without treatment to serve as controls

(Saatian et al., 2013). All samples were fixed and visualized using fluorescently tagged antibodies for tight junction proteins (e.g. claudins) and junction stabilizing proteins (e.g. occludins). Following a duplicate exposure to antibodies, the targeted proteins were observed using a laser scanning confocal microscope (Saatian et al., 2013).

To further specify the mechanism by which cytokine activity disrupted tight junctions, researchers focused on an intermediary enzyme involved with this pathway. Particularly, since previous studies have supported the idea that pro-inflammatory cytokines play a significant role in regulating MMP9 expression levels, researchers focused on what effects this specific protease has on the airway epithelium tight junctions (Vermeer et al., 2009).

Researchers used a human airway epithelia model which was evidenced to function comparably to in vivo structure and function. This model was produced from epithelium isolated from human trachea and bronchi samples that were seeded on a semipermeable membrane coated with collagen. Samples were grown for 14 days at 37°C in humidified conditions before being assayed (cultures required this time and these conditions to differentiate into ciliated epithelial cells) (Vermeer et al., 2009).

An ohmmeter was used to test transepithelial electrical conductance, where an increase in conductance relative to the control signifies weakened tight junctions. MMP9 was activated with p-aminophenylmercuric acetate then applied to the experimental epithelial cultures. Samples were labeled, analyzed and imaged using sphingomyelin-BODIPY labeling. This method functions off of the basis that since this label cannot

diffuse past tight junctions, properly functioning epithelium should exhibit restricted labeling of the basolateral membrane. To test resistance to viral infection, some cultures were infected with a fluorescently tagged adenovirus (whose receptor is located on the basolateral membrane) after being treated with active MMP9. Distribution of tight junction proteins (claudin and occludin) was assessed using immunocytochemistry using anti-claudin-1, anti-occludin, and anti-zonula occludens-1 antibodies from rabbits. This assay was visualized via ethidium bromide stain. To investigate cell loss as a result of MMP9 activity, cells disconnected from the epithelium one day after MMP9 treatment were washed and quantified (Vermeer et al., 2009).

After determining significant effects of MMP9 activity on tight junction formation, more studies were completed to further detail mechanisms involved. For instance, the change in phenotype of the epithelial samples treated with MMP9 (larger, fewer cells) compared to controls (smaller, more numerous cells), led the researchers to analyze apoptotic activity. Specifically, to illustrate this theory, samples were stained using caspase-3 antibody (since caspases play a significant role in apoptosis). In order to determine what mechanism resulted in apoptosis, researchers then analyzed cell activity of both control and experimental samples labeled for caspase and zona occludens-1 over time (imaged every 3 minutes for 30 hours) (Vermeer et al., 2009).

RESULTS

Transmission electron microscopy of the bronchial tissues of asthmatic patients were significantly different from that of normal patients. In normal subjects, tight

junction proteins (specifically occludin and ZO-1) were regularly distributed between columnar cells, concentrated towards the apical surface (Xiao et al., 2011). However, in asthmatic subjects, these proteins no longer exhibited a regular distribution; instead they were malformed, of varied size, or even missing completely (Figure 1). Even more significant, these irregular patterns were observed in a wide range of asthma patients from those diagnosed with mild to severe asthma and even both in the presence and absence of treatment (Xiao et al., 2011).

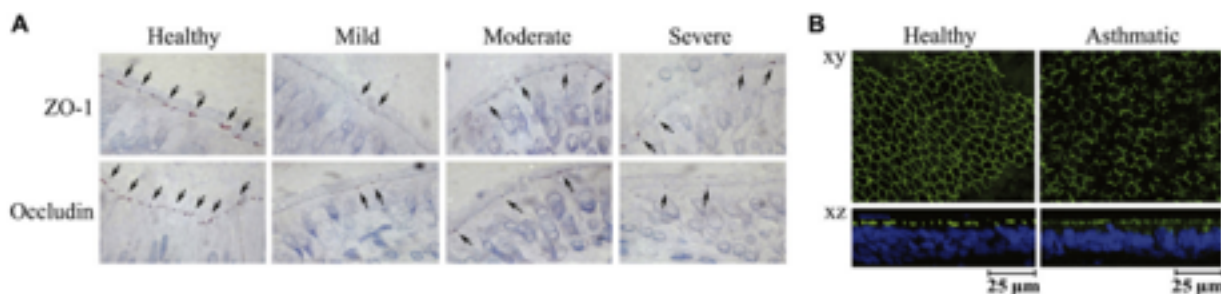


Figure 1. Comparison of distribution and abundance of tight junction proteins ZO-1 and occludin among healthy individuals and patients of varying degrees of asthma. 2-micrometer sections of bronchial biopsies obtained from both healthy and asthma affected individuals were fixed and ZO-1 and occludins were immunohistochemically stained and imaged using confocal microscopy (Xiao et al., 2011).

Researchers found that the *in vitro* addition of interleukins to epithelial tissue resulted in elevated permeability of those cells (Capaldo et al., 2008). These researchers report that this increased permeability is directly associated with and likely the result of decreases in amount of tight junction protein produced as a result of depressed mRNA expression in asthma patients (Capaldo et al., 2008). In turn, the depressed numbers of tight junction proteins were found to be attributable to cytokine activity (Figure 2) (Saatian et al., 2013).

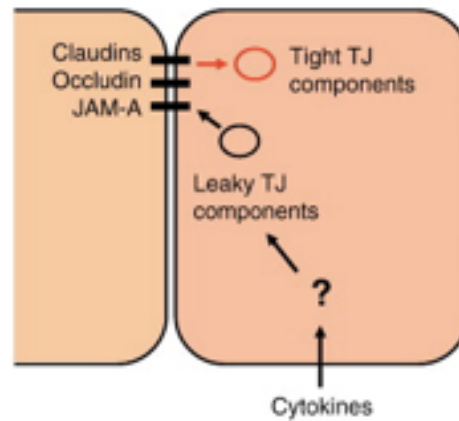


Figure 2. Increased permeability of airway epithelial tissue due to cytokine activity. By some unknown cellular mechanism, cytokine activity, results in an alteration of tight junction structure and thus function (Capaldo et al., 2008).

The key mechanism proposed connecting cytokine activity and tight junction protein disruption was found to be over-expression of MMP9. MMP9 treated cultures exhibited increased conductance similar to cultures treated with EGTA—a chelating agent that disrupts cell junctions. Sphingomyelin-BODIPY labeling demonstrated that MMP9 treated samples exhibited fluorescence in the basolateral membrane. Moreover, MMP9 treated samples demonstrated higher levels of infection by a virus (where the corresponding receptor is located on the basolateral membrane) than did non-treated samples (Figure 3) (Vermeer et al., 2009).

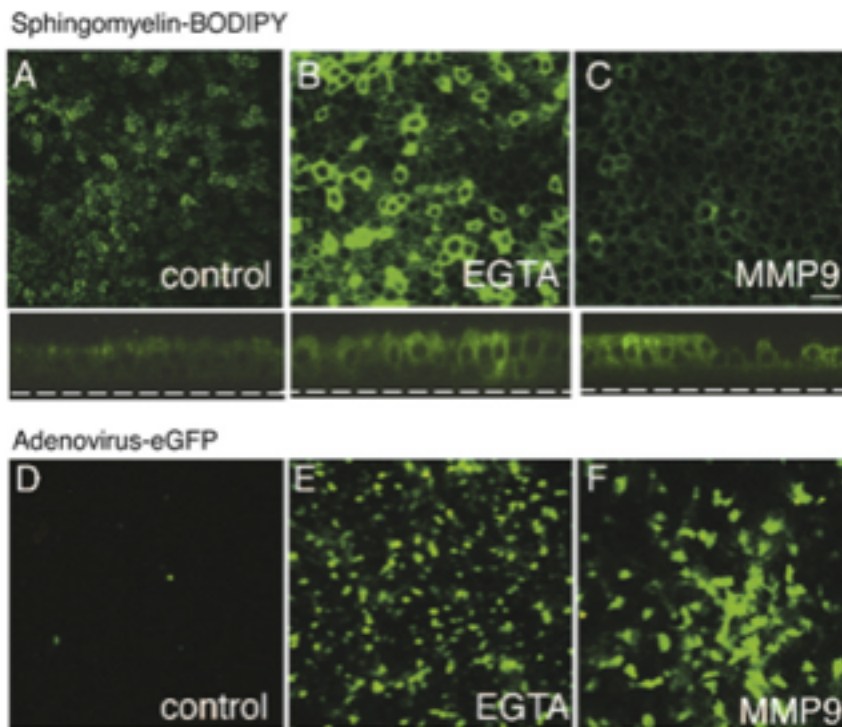


Figure 3. GFP labeled basolateral membranes of untreated, EGTA, and MMP9 treated human epithelial cultures.

Sphingomyelin-BODIPY does not label the basolateral membrane of untreated cultures, but does penetrate past tight junctions to label the basolateral membrane of MMP9 treated culture in a way similar to that of EGTA treated cultures (where EGTA directly breaks down cell-cell junctions). Similarly, MMP9 treated cultures exhibit increased infection since the virus could more easily penetrate the tight junctions in order to reach the corresponding receptor on the basolateral membrane (Vermeer et al., 2009).

Analysis of the immunocytochemistry assay used to visualize localization of tight junction proteins occludin and claudin-1 demonstrated an altered distribution pattern of these proteins. MMP9 treated samples also consisted of fewer and larger sized cells when compared to control. Even after taking such changes into account, MMP9 treated samples demonstrated a decrease in the total number of cells as well as an increased number of disconnected cells (Figure 4) (Vermeer et al., 2009).

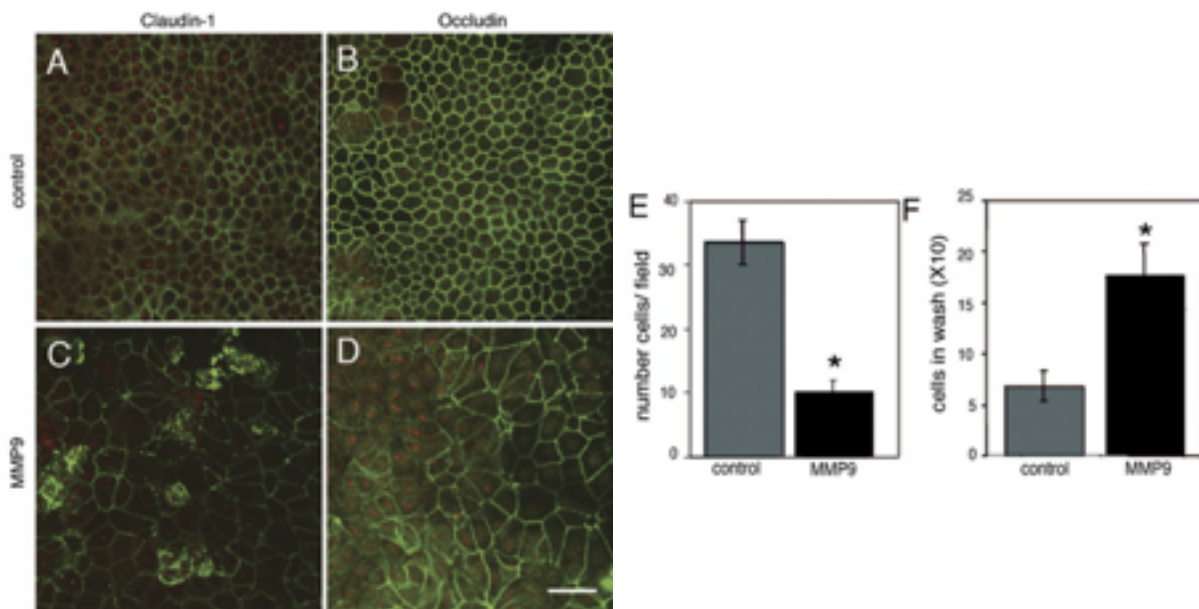


Figure 4. Comparison of distribution of claudin-1 and occludin tight junction proteins between untreated and MMP9 treated tissue samples as well as comparison of cells present within continuous sample and those detached from sample.

MMP9 treated samples consisted of fewer tight junction proteins as well as fewer and larger cells when compared to control samples. MMP9 samples also exhibited a more numerous population of disconnected cells (Vermeer et al., 2009).

Staining with caspase-3 antibody demonstrated that apoptosis was unregulated in MMP9 treated cells in comparison to control samples. In fact, MMP9 treated samples demonstrated a phenotype similar to that of samples treated with staurosporine (which directly induces apoptosis). The 30 hour lapse of images demonstrated few changes in control images over time. However, MMP9 treated samples exhibited cell extrusion that resulted in fewer and larger cells (Figure 5) (Vermeer et al., 2009).

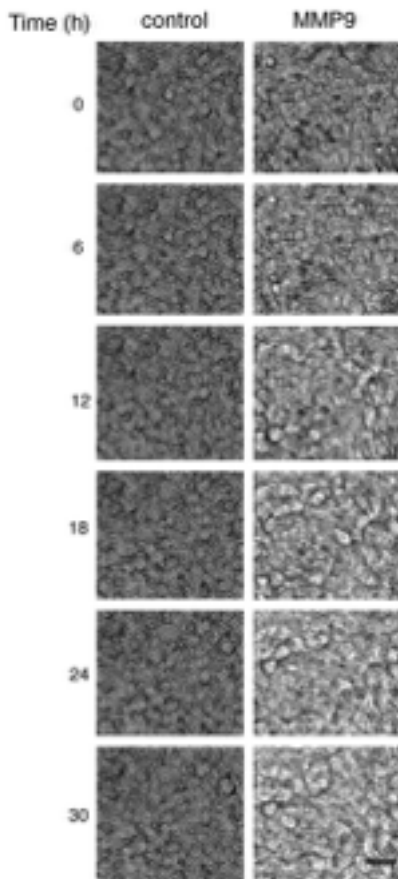


Figure 5. Comparison of growth between control and MMP9 treated samples over a 30 hour time period.

Control samples appear to alter little in appearance over time. However, MMP9 treated samples appear to consist of fewer, larger cells over time. This change in phenotype is likely due to excessive cell extrusion where cells that are lost are replaced by fewer larger cells and thus results in a net loss in the amount of cells but not in total surface area (Vermeer et al., 2009).

DISCUSSION

The significant differences of both quantity and distribution of tight junction proteins observed between normal patients and a number of varying asthmatic patients indicates that tight junction distribution is a potential underlying mechanism for all subcategories and degrees of asthma. While there was a statistically significant difference

in protein levels between healthy individuals and asthma affected individuals, there was no difference in expression levels of mRNA for either of these proteins. Thus, it is likely that a post-transcriptional mechanism is responsible for the dissimilarity (Xiao et al., 2011).

This observation can be explained by connecting tight junction disruption to cytokine activity where MMP9 is the main intermediary. The fact that MMP9 treated cultures exhibited increased conductance similar to cultures treated with EGTA—a chelating agent that disrupts cell junctions—provides evidence that overactive MMP9 expression damages cell junctions (Vermeer et al., 2009). Observations from Sphingomyelin-BODIPY labeling (MMP9 treated samples exhibited fluorescence in the basolateral membrane) signify that the label was able to penetrate the tight junction, further supporting the conclusion that tight junctions are compromised. Moreover, similarly, the fact that the MMP9 treated samples demonstrated higher levels of infection compared to controls again supports the idea that tight junctions must have been disrupted in order for the virus to access the corresponding receptor (since the viral receptor is located at the basolateral membrane) (Vermeer et al., 2009). Alterations in the distribution and number of tight junction proteins occludin and claudin-1 as well as the characterization of fewer and larger sized cells in samples treated with MMP9 signify altered development and maintenance of epithelium in the presence of over-expressed MMP9. Specifically, these alterations involve critical changes on the regulation of cell apoptosis—MMP9 treated samples exhibited excessive cell extrusion. Normally, cell

extrusion occurs to relieve overcrowding of cells; however, in this case, not enough new cells are produced to maintain a homeostatic number of cells, resulting in fewer and larger cells occupying the same surface area. In other words, MMP9 targets components at the tight junction which results in detachment-induced cell death (anoikis) (Vermeer et al., 2009).

Further, this mechanism supports the main theorized pathway behind the chronic manifestation of asthmatic symptoms and clinical features in human patients. In asthmatic patients, these disrupted tight junctions and epithelial cells do not undergo an adequate repair response (Holgate, 2008). In individuals with healthy airway epithelium, damage to this layer caused by any variety of factors (such as that due to inhaled particles, bacteria, or viruses) is responded to by means of epidermal growth factor (EGF) (Holgate, 2008). This growth factor participates in repair by encouraging cell migration to the site of damage and promoting cell proliferation once at this location. However, asthma patients experience a reduced proliferation in response to such damage, presenting a possible explanation for the chronic characterization of asthma (Holgate, 2008). Examination of epithelium repair in asthma patients was fulfilled by studying levels of cell cycle markers (Ki67 which correlates with DNA synthesis) and inhibitors (p21^{waf}). Levels of cell cycle markers should thus correlate directly with rates of proliferation while levels of cell cycle inhibitors should correlate inversely with rates of cell proliferation (Holgate, 2008). Researchers collected mucosal specimen from both healthy and asthma-affected individuals via bronchoscopy; samples were analyzed using

immunohistochemistry. Specifically, they were stained using anti-EGFR, anti-p21, and anti-Ki67 antibodies (Fedorov et al., 2005).

Bronchoscopies illustrated statistically significant increases in the levels of EGFR and p21^{waf}, but reduction of Ki67 levels in asthma patients (Holgate, 2008). The observed increases in the levels of EGFR and p21^{waf}, as well as the reduction of Ki67 levels in asthma patients, support the idea that asthma is characterized by compromised repair mechanisms (Holgate, 2008). While the levels of EGFR correspond to a properly initiated immune response, the elevated levels of the cell cycle inhibitor p21^{waf} as well as reduction of the cell cycle marker Ki67 demonstrate inhibited proliferation of cells at the site of airway tissue damage. Such inhibition is a confirmation of ineffectual repair in asthma patients. This inability of the airway epithelium to properly heal results in a persistently compromised respiratory system, further aggravating the already damaged epithelium, and is thus likely the mechanism behind the chronic characterization of asthma.

The current theory of the manifestation of asthma combines both genetic and environmental factors which contribute to a chronic illness which can be exacerbated at times dependent upon mainly environment. In general, the popular hypothesis is that asthma results due to tight junction disruption. This disruption is a result of cytokine activity that up regulates expression of matrix metalloprotease 9. MMP9 encourages detachment-induced cell death—anoikis—which results in a structural change compromising the airway epithelium's ability to act as a primary immune defense

mechanism. The chronic characterization of asthma results because of an ineffective epithelial repair mechanism which prevents full recovery of the affected airway tissues thus allowing for further aggravation by environmental pathogens.

Chapter 3

Asthma: A Clinical Case Study

Patient Introduction

Meredith Kurtzman is a 52-year-old, widowed, hispanic female who presents with dyspnea, wheezing, and coughing (AAFA, 2016). It has been one week since her most recent hospitalization.

Chief Complaint

“I have been experiencing trouble breathing and cannot stop coughing.”

History of Present Illness

Meredith presents today for follow up after she was hospitalized one week ago. She sought emergency medical attention after experiencing unremitting, nonproductive coughing and wheezing as well as shortness of breath. Upon presentation to the emergency department, she was found to be hypoxic with an O₂ saturation of 89%, tachycardic with a heart rate of 120 beats per minute, and tachypneic with a respiratory rate of 24 breaths per minute. She was diagnosed with influenza and was treated initially with 5 liters of oxygen. This did not resolve the patient’s tachypnea and the patient mentioned that, as a child, she had previously used a peer’s inhaler to great effect during a similar episode. She was subsequently given nebulized albuterol and oral glucocorticoids. Meredith was admitted to the inpatient ward where her symptoms resolved over the next two days. She was discharged with a tapered prednisone dose pack and a short-acting beta-agonist inhaler to use as needed (Volovitz, 2003). She was told to

follow up with her primary care physician for a long-term treatment plan. Since her hospitalization a week ago, she has been using the inhaler approximately two to four times everyday. However, the last dose she took prior to her appointment this afternoon, was yesterday morning. She expressed that she wanted to be seen with the symptoms that she had been experiencing leading up to the hospitalization. Meredith says that for about one to two weeks leading up to her most recent hospitalization, she was ill with an upper respiratory infection. She experienced coughing, wheezing, and shortness of breath (Patadia, 2014). Throughout adulthood, Meredith has experienced chronic coughing and wheezing with intermittent flare ups similar in severity to those symptoms she experienced just prior to seeking emergency medical attention. She has been hospitalized two times as an adult (last week as well as a few months ago).

Meredith stated that she has suffered from this chronic nonproductive coughing and wheezing since she was a child (ACAAI, 2014). Growing up, these symptoms were particularly exacerbated each time she fell ill with the flu or when her seasonal allergies flared during the spring. She noted that when she has colds, she suffers more severe symptoms that last noticeably longer compared to her brother. She had been hospitalized several times throughout childhood for upper respiratory infections. As a child, a peer offered her an inhaler, and she remembers experiencing a temporary but noticeable improvement in her symptoms after use. She also stated having frequent rashes, consistent in description to eczema, on the insides of her elbows and occasionally behind her knees as well (Patadia, 2014). Due to Meredith's low socioeconomic status, she did

not have access to medical care and these rashes were treated with home remedies. As Meredith has never established a regular medical provider, there has been a lack of communication concerning her medical history. Meredith is here today as she desires a firm diagnosis.

Review of Systems

Meredith denies fever/chills, headache, dizziness, changes in vision, changes in hearing, sore throat, nausea/vomiting, chest pain, abdominal pain, dysuria, changes in urinary frequency, constipation/diarrhea, changes in weight, swelling in her limbs.

Social and Family History

Meredith was raised in Phoenix, Arizona with her parents, Joanna and Frank, and her older brother, Craig. Meredith finished high school but did not seek higher education. Throughout middle school and high school, Meredith experienced a number of sports-related injuries—a sprained ankle and pulled hamstring from cheerleading. She noted that she always felt more winded and required more breaks compared to the other girls on the team.

Meredith lived with her parents and worked at a retail store until age 21 when she married Kent, a small business owner. She had two children with her husband at 35 and 37. Her husband passed in a car accident 5 years ago, so she has since been the primary caretaker of her two children. While married she was unemployed, but now she works as a maid and feels like she has no time to take care of herself. Although she is very tired by night, she still has major issues sleeping through the night, expressing frequent (nightly)

nighttime awakenings. She gets about 4 hours of sleep each night, frequently interrupted by wheezing and difficulty breathing (Patadia, 2014). She is not dating and is not sexually active.

Her parents, Joanna and Frank, are 79 and 83, respectively. Joanna is healthy and very active and Frank has high cholesterol. None of Meredith's grandparents are still living, but they were all relatively healthy, passing in their 90's, except for her paternal grandfather, a smoker who passed at age 76 from lung cancer. Meredith's older brother, 56, also has eczema on the inside of his elbows.

Meredith has a history of chronic coughing, wheezing and eczema. She is up to date on her immunizations except for the flu shot which she did not receive this year. Major surgeries include wisdom teeth removal at 19 and a C-section at 37. Meredith has no known food allergies, but has a history of allergies to pet dander, especially that of cats. She rarely drinks socially, stating that she never has more than one or two drinks a month, and she denies use of nicotine and other recreational drugs.

Vital Signs:

Height: 5'5"

Weight: 128 lbs

BMI: 21.3 (normal)

Body Temperature: 98.6°F

Pulse: 112 BPM

Respiration rate: 20 breaths/min

Blood Pressure: 100/70mmHg

O₂ Saturation: 92% (low)

Physical Exam

General: 52 yo female appears tired and short of breath. She is alert and cooperative.

Head: Normocephalic and atraumatic.

Eyes: Extraocular movement intact. Pupils equal, round and reactive to light and accommodation. Wears corrective lenses.

Ears: Clear external auditory canals and tympanic membranes. Hearing intact.

Throat/Oral: Post-nasal drip, inflammation of oropharynx.

Neck: No lymphadenopathy. Negative Brudzinski and Kernig tests.

Pulmonary: Wheezing during forced exhalation. No rhonchi or rales heard on auscultation.

CVS: Increased pulse rate, Regular rhythm. No murmurs, rubs or gallops. Normal S1 and S2. Non-displaced point of maximal impulse.

Abdominal: Soft, nontender, and nondistended in all quadrants. Normal bowel sounds. No palpable masses.

Rectal Exam: No anal, perianal, or rectal lesions, fistulas, or fissures.

Musculoskeletal: No tenderness, spasms, or deformities. No joint pain or erythema. Normal range of motion and alignment.

Extremities: No tenderness, edema, or varicosities. No cyanosis or clubbing. No deformities or joint abnormalities. Strength is 5/5 in all extremities.

Skin: Normal color, turgor and temperature. Erythematous and vesicular crusty patch noted on inside of elbows.

Neurological: Alert and oriented. CN II-XII grossly intact. Face is symmetric, no facial droop or nystagmus. Strength, sensation and reflexes normal.

Diagnostic Work

Test	Result	Reference Range
Complete Blood Count (CBC)		
Red Blood Cell (RBC)	5.0x10 ⁶ /μL	4.2-5.9x10 ⁶ /μL
Hemoglobin (HB/Hgb)	14 g/dL	12-16 g/dL
Hematocrit (HCT)	41%	36-47%
Mean Cell Volume (MCV)	90 fL	80-100 fL
Mean Cell Hemoglobin (MCH)	34 g/dL	32-36 g/dL
White Blood Cell (WBC)	10,000 /μL	4000-10,000 /μL
Platelets	220,000 /μL	150,000-350,000 /μL
WBC Differential		
Neutrophil (Absolute)	5500/μL	
Neutrophil (Relative)	55%	33-73%
Lymphocyte (Absolute)	3400/μL	
Lymphocyte (Relative)	34%	13-52%
Monocyte (Absolute)	500/μL	
Monocyte (Relative)	5%	0-10%
Eosinophil (Absolute)	500/μL	
Eosinophil (Relative)	5%	0-5%
Basophil (Absolute)	100/μL	
Basophil (Relative)	1%	0-2%

Pulmonary Function Tests						
Spirometry	Ref	Pre-Bronchodialator	% Predicted	Post-Bronchodialator	% Predicted	% Chg
FVC	2.84 L	2.3 L	81%	2.39 L	84%	3.9%
FEV1	2.32 L	1.28 L	55%	1.67 L	72%	30.5%
FEV1/FVC	81.6%	56%	n/a	70%	n/a	n/a

(UpToDate, 2016)

Differential Diagnosis

Chronic bronchitis was considered because symptoms include shortness of breath and wheezing and affected individuals exhibit partial improvement of symptoms when given asthma treatment (such as albuterol). The major cause of this illness is cigarette smoking (more than 10 pack-years); however, Meredith has no history of smoking. Also, chronic bronchitis is characterized by a productive cough. While Meredith has exhibited a chronic cough since childhood, it was noted as nonproductive. Thus, chronic bronchitis was deemed unlikely.

Similarly, chronic obstructive pulmonary disease (COPD) was considered due to Meredith's symptoms of shortness of breath and wheezing. However, again, COPD is largely due to heavy cigarette smoking (more than 20 pack-years). Also, individuals with COPD do not generally exhibit an improvement in FEV1 value (following treatment with bronchodilator) as significant as that exhibited by Meredith (30.5% improvement). In fact, any improvement seen in COPD patients following bronchodilator treatment generally does not result in normal values. Also, the ratio of FEV1/FVC appears almost

normal for individuals with COPD (since both their FVC and FEV1 values are low) compared to asthmatic patients whose ratio is much smaller (since their FVC value is almost normal while their FEV1 value is low). Meredith exhibited a much smaller FEV1/FVC ratio than predicted prior to treatment with bronchodilator (56%). Further, the first symptoms of COPD do not generally present until about age 35-40. Based on Meredith's history, her chronic symptoms (wheezing and coughing) likely began in childhood but went undiagnosed.

Heart failure was also considered since left sided heart failure presents with symptoms of dyspnea and trouble breathing at night. However, Meredith has a relatively healthy lifestyle—she denies nicotine use and excessive alcohol consumption and is not overweight. Also, heart failure is not commonly found in individuals under the age of 60. Her physical exam further supports that she is not affected by heart failure—normal S1 and S2, no murmurs, no edema in the lower extremities and belly non-distended (symptoms of right sided heart failure), and normal point of maximal impulse (symptom of an enlarged heart).

Lung cancer was also considered since it would result in symptoms of persistent cough and shortness of breath. However, compared to Meredith's cough which was non-productive, lung cancer patients tend to cough up blood or reddish sputum. Further, Meredith did not exhibit any weight changes or systemic effects that most cancer patients are affected by.

Constrictive bronchiolitis was also considered since symptoms include shortness of breath due to narrowing of the bronchioles. However, this illness is highly characterized by crackling sounds in the lungs. No crackles, also referred to as rales, were observed during Meredith's physical examination. Further, constrictive bronchiolitis is an irreversible airflow limitation. Thus, individuals affected by constrictive bronchiolitis would not exhibit improvement in pulmonary function when treated with a bronchodilator. Since Meredith exhibited a significant improvement in FEV1 value, this further supports ruling out constrictive bronchiolitis.

Allergic rhinitis was also considered due to Meredith's chronic cough. Individuals affected with allergic rhinitis exhibit allergic symptoms in response to certain allergens. However, allergic rhinitis is frequently comorbid with asthma. For instance, since Meredith exhibits allergies to cat dander, it is likely that Meredith exhibits allergic rhinitis. While this may exacerbate her symptoms, it is unlikely the root cause of them since Meredith also experiences severe shortness of breath.

Post-viral tussive syndrome was considered since Meredith was diagnosed with influenza during her hospitalization. This cough can last for up to eight weeks following a viral respiratory tract infection. While this illness is a possibility, it is unlikely given Meredith's history of present illness. The fact that she has experienced a persistent coughing and wheezing since childhood and has been affected by frequent cases of eczema support a diagnosis of asthma over post-viral tussive syndrome.

Diagnosis and Supporting Argument

Meredith's history, physical exam, and diagnostic findings are consistent with asthma. As a child, Meredith experienced chronic coughing and wheezing. Such symptoms were so severely exacerbated during upper respiratory infections and seasonal allergies that she was hospitalized several times as a child. This pattern is consistent with asthma. Meredith's admission to the use of an inhaler which resulted in an improvement in her symptoms further supports the idea that Meredith has suffered from asthma since childhood. Further, both Meredith and her brother were affected with eczema which is frequently comorbid with asthma. While her childhood symptoms appear to be highly consistent with asthma, Meredith probably went undiagnosed due to her family's financial inability to establish a regular medical provider.

The fact that her symptoms of wheezing and coughing have persisted since childhood and have even resulted in two adult hospitalizations support the idea that Meredith suffers from asthma which may have even increased in severity since childhood. For instance, her frequent nighttime awakenings are also consistent with severe asthma. Meredith's use of the short-acting beta-agonist inhaler from the emergency department approximately two to four times everyday since her hospitalization is similarly indicative of severe asthma. During her physical exam, she exhibited elevated respiratory and heart rates, supporting the diagnosis of asthma since affected individuals must compensate for decreased oxygen intake with a more rapid intake and delivery of oxygen through the circulatory system. Examination of Meredith's nose, throat and neck further support the diagnosis of severe asthma as she exhibited

increased nasal secretions, inflammation of oropharynx, mucosal swelling, and post-nasal drip. Examination of her skin resulted in observation of erythematous, vesicular crusty patches consistent with eczema which, again, is frequently comorbid with asthma.

Meredith's blood tests show that all white blood cells are present within normal values (Lab Tests Online, 2016). Her spirometry test showed a FEV1/FVC value of less than 70% (56%, which is indicative of an obstructive process such as asthma), an FEV1 value of less than 60% predicted (55%, which is indicative of severe asthma), and at least a 12% increase in FEV1 following use of a bronchodilator (30.5% increase, also indicative of severe asthma) (Johnson, 2014). Meredith's improvement in FEV1 is indicative of an obstructive (narrowed air passages) lung disease such as asthma. On the other hand, her FVC value was normal, indicating that she is likely unaffected by a restrictive (inability to fully fill lungs) lung disease (UpToDate, 2016).

Further, more than 50% of asthma cases in the U.S. are comorbid with allergies. Cat allergies are the highest allergen predictor of asthma risk so her allergy to cats further supports the diagnosis of asthma (Patadia, 2014).

Treatment Plan

The main focus of Meredith's treatment plan is managing her asthma symptoms to improve her quality of life (National Heart, Lung, and Blood Institute, 2014). This includes relieving the tightness in her chest, wheezing, and coughing as well as her nighttime awakenings. The patient should be treated with an inhaled corticosteroid which reduces the inflammation of the airways and thus alleviates her symptoms of tightness in

chest, wheezing and coughing (UpToDate, 2016). She was originally prescribed with a medium dose (one puff of 250 mcg per puff twice daily) of the inhaled corticosteroid fluticasone propionate. There are several other inhaled corticosteroids on the market (including beclomethasone, budesonide, ciclesonide, and mometasone). If her symptoms have not been managed after a month of use, a higher dosage (two puffs of 250 mcg per puff twice daily) should be prescribed. If her symptoms are still not alleviated, different corticosteroids should be prescribed.

Supplementing these inhaled corticosteroids with long-acting beta-agonists can further alleviate asthma symptoms (UpToDate, 2016). In particular, these beta-agonists can help relieve nighttime asthma symptoms and reduce the risk of exacerbation of symptoms. They are, in turn, made more potent by inhaled corticosteroids. She was prescribed with one inhalation of the long acting beta agonist Salmeterol (Serevent Diskus) once every 12 hours (each inhalation contains 50 mcg). If Meredith does not note any progress on this beta-agonist after a month of use, she could either try another type of long-acting beta-agonist (formoterol). Meredith was also prescribed a short-acting beta-agonist inhaler—a rescue inhaler—and 20 mg tablets of prednisone to use as needed.

There are inhalers which have combined corticosteroids and long-acting beta-agonists and are thus easier to use; however, these inhalers (e.g. Symbicort or Advair) tend to be much more expensive and thus are likely unfeasible for a patient of low socioeconomic status such as Meredith. As Meredith requires step 4 level treatment, she will need to be referred to a pulmonary specialist.

Meredith was given an asthma action plan and a peak flow meter in order to monitor her peak expiratory flow rate (Srinivas, 2015). This action plan will help her monitor her symptoms and adjust her medication based on her peak expiratory flow rate and other symptoms in order to prevent severe episodes. The action plan defines green, yellow, and red zones based on peak expiratory flow rate (Meredith's personal predicted peak expiratory flow rate is approximately 420 liters per minute) and symptoms and lists recommended treatment for each zone (NAU, 2016, University of Michigan, 2016). A green zone is characterized by a peak flow of greater than or equal to 80% predicted, few to no symptoms, and the ability to complete normal daily activities. Medication to be taken daily under this condition include one puff of 250 mcg per puff fluticasone propionate twice daily and one puff of 50 mcg per puff salmeterol every 12 hours. A yellow zone is characterized by a peak flow of 50-79% predicted, symptoms (such as coughing, wheezing, shortness of breath, fatigue), and inability to complete normal daily activities. Under these conditions, Meredith should take one to two treatments (20 minutes apart) of 2-6 puffs of her short-acting beta-agonist inhaler. Also, in addition to her green zone medications, Meredith should also take a single dose of two 20 mg tablets of prednisone (an oral glucocorticoid). This should be continued daily until her peak expiratory flow rate returns to at least 80% predicted (generally about 3-10 days of treatment). A red zone is characterized by a peak flow of less than 50% predicted accompanied by severe symptoms (same symptoms as yellow zone but increased in severity). Under this condition, Meredith should take one to two treatments (20 minutes

apart) of 2-6 puffs of her short-acting beta-agonist inhaler. Also, Meredith should take a single dose of three 20 mg tablets of prednisone daily on top of her green zone medications until her peak expiratory flow rate returns to 80% predicted. If peak flow and symptoms still do not improve, she should go to the emergency department.

Prognosis

There is currently no cure for asthma. With proper treatment, remission is rare but possible. However, remission is generally seen only in the most mild cases of asthma. Since Meredith suffers from severe asthma, she is unlikely to undergo complete remission. Unfortunately, without proper care and observation, cases such as Meredith's often result in the development of permanent lung damage over time (Lange, 1999). With proper treatment, Meredith should be able to lead a normal life. While establishing the proper treatment plan, Meredith should visit her physician every two to four weeks. Once her symptoms have been successfully controlled, Meredith will require checkups every six months to maintain the current health of her lungs and control of her symptoms as well as to investigate any progression of further lung damage. However, if Meredith notices her symptoms worsening or becoming more frequent despite proper usage of medication, she should visit her physician sooner. As Meredith requires step 4 level treatment (a medium-dose inhaled glucocorticoid and a long-acting beta-agonist), she will also need to be referred to a pulmonary specialist.

Complications of asthma include difficulty exercising and sleeping, permanent lung damage, and persistent symptoms (coughing and wheezing). Also, mortality rates

double for asthma patients due to increased susceptibility of lung disease (National Asthma Control Initiative, 2016). Complications and mortality are less probable with recommended use of inhaled corticosteroids as they maintain lung function level (MedlinePlus, 2016).

Plan Implementation

Meredith will need to visit with her primary physician every two to four weeks until she establishes a treatment plan that properly controls her symptoms. As Meredith requires step 4 level treatment, she will also need to be referred to a pulmonary specialist. Once her medications appear consistently effective, she should continue to follow up with her physician every six months to monitor her asthma. Meredith should continue to utilize her asthma action plan to modulate her medication based on her symptoms.

Since Meredith works around many household allergens as a maid, it is recommended that—once she feels well enough to return to work—she wear an allergy mask, especially when working with cleaning products and dusting. Preventing these allergens from entering her respiratory system will prevent the risk of symptom exacerbation. Further, it is recommended that Meredith look into an over the counter antihistamine, such as Allegra or Zyrtec, to relieve her seasonal allergies.

Since asthma treatment is life-long and continuous, this illness may add additional stress to Meredith's life. For instance, the cost of medication may further contribute to Meredith's financial stress. Taking into account that Meredith is of low socioeconomic status, the initial medicines prescribed are aimed to minimize cost while still maximizing

effectiveness. Further, in 2013, the Patient Access Network (PAN) Foundation began a financial assistance program for asthma patients. If Meredith fits the financial requirements (income below 500% of the Federal Poverty Level and expenses greater than \$100/month), she could receive \$3000 annually to go towards her asthma medications. More information on this program can be sought at www.PANfoundation.org (PANFoundation, 2016). Another stress Meredith may encounter is finding a support group. While her husband has passed, Meredith still lives relatively close to her parents and brother in Phoenix and has many friends who can support her.

References

- American College of Allergy, Asthma, and Immunology. (2014). "Asthma." *ACAAI*.
- Asthma. (2015, October 1). Mayo Clinic.
- Asthma and Allergy Foundation of America. (2016). "Asthma." *AAFA*.
- Bernstein, J. (2014). *Clinical asthma theory and practice*. Boca Raton: CRC/Taylor and Francis.
- Bossé, Y., & Hudson, T. (2007). Toward a Comprehensive Set of Asthma Susceptibility Genes. *Annual Review of Medicine*, 58, 171-184.
- Capaldo, C., & Nusrat, A. (2008). Cytokine regulation of tight junctions. *Biochimica Et Biophysica Acta*, 1788(4), 864-871.
- Diette, G.B., McCormack, M.C., Hansel, N.N, Breyse, P.N., Matsui, E.C. (May 2008). Environmental Issues in Managing Asthma. *Respiratory Care*, 53(5), 602-617.
- EM Sample Preparation Contrasting* (pp. 1-17). (2013). Vienna: Leica Mikrosysteme.
- Fedorov, I. (2005). Epithelial stress and structural remodelling in childhood asthma. *Thorax*, 60(5), 389-394.
- Goolsby, M., & Judd, D. (n.d.). Guidelines for the Diagnosis and Management of Asthma: Update on Selected Topics 2002. *Journal of the American Academy of Nurse Practitioners*, 151-155.
- Holgate, S. (2008). Pathogenesis of Asthma. *Clinical and Experimental Allergy*, 38(6), 1608-1631.
- Johns, D.P., Pierce, R. (2008). The Measurement and Interpretation of Ventilatory Function in Clinical Practice. *Spirometry*.
- Johnson, J.D. and Theurer, W.M. (2014). "A stepwise approach to the interpretation of pulmonary function tests." *American Family Physician*, 89(5), 359-366.
- Khalid, I., Obeid, I., Digiovine, B., Khalid, U., & Morris, Z. (2009). Predictive Value of sGaw, FEF 25 – 75 , and FEV 1 for Development of Asthma after a Negative Methacholine Challenge Test. *The Journal of Asthma*, 46(3), 284-290.

- Kudo, M., Ishigatsubo, Y., & Aoki, I. (2013). Pathology of asthma. *Frontiers in Microbiology*, 4, 263-263.
- Lab Tests Online. (2016). "WBC Differential." *LabTestsOnline*.
- Lange, P. (1999). "Prognosis of adult asthma." *PubMed*, 54(4), 350-2.
- Mcneil, E., Capaldo, C., & Macara, I. (2006). Zonula Occludens-1 Function in the Assembly of Tight Junctions in Madin-Darby Canine Kidney Epithelial Cells. *Molecular Biology of the Cell*, 1922-1932.
- MedlinePlus. (2016). "Asthma." *U.S. National Library of Medicine*.
- Montuschi, P., & Peters-Golden, M. (2010). Leukotriene modifiers for asthma treatment. *Clinical & Experimental Allergy*, 40(12), 1732-1741.
- Mysore, S., & Ruffin, R. (2011). Long-Acting β -Agonists in Asthma Management. *Drugs*, 71(16), 2091-2097.
- National Asthma Control Initiative. (2016). "Asthma." *U.S. Department of Health and Human Services*.
- National Heart, Lung, and Blood Institute. (2014). "Asthma." *NIH*.
- Northern Arizona University. (2016). "Pulmonary Function Testing." *NAU*.
- Patadia, M.O., Murrill, L.L & Corey, J. (2014). "Asthma." *ClinicalKey*, 47(1), 23-32.
- Patient Access Network Foundation. (2016). "Asthma: Get Help with your Treatment." *PAN Foundation*.
- Peden, D. (2000). Development of Atopy and Asthma: Candidate Environmental Influences and Important Periods of Exposure. *Environmental Health Perspectives*, 108(3), 475-482.
- Ratageri, V. (2001). Brief report. Lung function tests in asthma: Which indices are better for assessment of severity? *Journal of Tropical Pediatrics*, 57-59.
- Saatian, B., Rezaee, F., Desando, S., Emo, J., Chapman, T., Knowlden, S., & Georas, S. (2013). Interleukin-4 and interleukin-13 cause barrier dysfunction in human airway epithelial cells. *Tissue Barriers*, 1(2), E24333.

- Schatz, M., & Camargo, C. (n.d.). The relationship of sex to asthma prevalence, health care utilization, and medications in a large managed care organization. *Annals of Allergy, Asthma & Immunology*, 553-558.
- Sobande, P., & Kerckmar, C. (2008). Inhaled Corticosteroids in Asthma Management. *Respiratory Care*, 53(5), 625-634.
- Srinivas, P. (2015). "Benefit of Asthma Action Plan." *Journal of Evolution of Medical and Dental Sciences*, 4(72), 12537-12541.
- Vercelli, D. (2008). Discovering susceptibility genes for asthma and allergy. *Nature Reviews Immunology*, 8, 169-182.
- University of Michigan. (2016). "Predicted Average Peak Expiratory Flow." *UMich*.
- UpToDate. (2016). "Asthma" *UpToDate*.
- Vermeer, P. D., Denker, J., Estin, M., Moninger, T.O., Keshavjee, S., Karp, P., Kline, J. N., Zabner, J. (2009). MMP9 modulates tight junction integrity and cell viability in human airway epithelia. *Lung Cellular and Molecular Physiology*, 296(5), 751-762.
- Volovitz, B. (2003). "Treatment of acute asthma attack in the emergency department." *PubMed*, 142(11), 750-3.
- What Are the Signs and Symptoms of Asthma? (Aug 2014). National Heart, Lung, and Blood Institute.
- Xiao, C., Field, S., Haywood, J., Broughton-Head, V., Bedke, N., Cremin, C., . . . Davies, D. (2011). Defective Epithelial Barrier Function In Asthma. *Mechanisms Of Airway Response To Injury*, 128(3), 549-556.
- Yassin, A., Aodah, A., Al-Suwayeh, S., & Taha, E. (2012). Theophylline colon specific tablets for chronotherapeutic treatment of nocturnal asthma. *Pharmaceutical Development and Technology*, 17(6), 712-718.