RESPIRATORY SYNCYTIAL VIRUS (RSV) AND ASTHMA—POTENTIAL FOR INTERVENTION

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Table of Contents

Respiratory Syncytial Virus (RSV) and Asthma – Potential For Intervention

Abstract

Respiratory Syncytial Virus (RSV) is a common virus that has the potential to infect people of all ages and is prevalent in countries all over the world. Most cases of RSV infections are presented in the upper respiratory tract, but there are cases of lower respiratory tract infection, which tend to lead to more harmful consequences. In some instances, RSV infections can be responsible for the emergence of other short-term respiratory issues, such as pneumonia and bronchiolitis. This can occur if an RSV infection worsens and leads to conditions that make the lungs susceptible to illnesses and even chronic diseases. Asthma is common chronic health condition affecting millions of people worldwide, and increasing evidence shows that having severe RSV infections at an early age is linked to adult asthma. This literature review focuses on research findings that provide supportive evidence for how RSV is linked to asthma and possible interventions for both RSV and asthma. I will look at the backgrounds, the pathophysiology, and treatments and preventative options of both RSV and asthma. Then, I will focus on the main findings among the correlations between the cytokines that are upregulated during severe RSV infections, such as interleukin-33 (IL-33), IL-13, and IL-5, and how those same cytokines are involved with asthma genesis and recurring asthmatic inflammation. Finally, I will discuss the potential for preventing asthma development caused by RSV via early RSV vaccination.

Introduction

Respiratory Syncytial Virus (RSV)

Respiratory syncytial virus (RSV) is one of the most widespread viruses seen in humans all over the world.[1] The virus can develop into either an upper respiratory infection or a lower respiratory tract infection which differ in the severity of symptoms.[1] When the virus leads to an upper respiratory infection, the symptoms are similar to those of a cold where individuals may experience a dry cough, a sore throat, a mild fever, and a congested or runny nose.[2] However, with severe cases of RSV infection, it can travel down to the lower respiratory tract and manifest symptoms which include labored breathing, severe coughing, wheezing a fever, and hypoxia that could lead to cyanosis in infants.[2] The surrounding environment plays a role in instigating RSV infections, as exposure to tobacco, being in a daycare, or having siblings or family members who may be susceptible to infection are controllable factors associated with increased RSV infection.[3] Tropical climates and winter seasons contribute to increased RSV infection rates, as well, with rates being the highest from November to April in the United States.[1]

While it is possible for anyone to contract RSV, the virus predominantly affects infants, the elderly, and individuals with compromised immune systems.[4] RSV is currently the major cause of lower respiratory tract infection (LRTI) and hospitalizations in children worldwide.[5] In a clinical setting, RSV is presented as an upper respiratory infection for most adults, yet in young children and infants, the infection often spreads to the lower respiratory tract where it can lead to other comorbidities, such as development of bronchiolitis or pneumonia.[1] A confounding factor of RSV is that infections are often harmful to those who are most at risk for contracting the infection and most at risk for developing severe symptoms. For example, those who suffer from compromised immune systems, such as premature infants, children younger than the age of two, and elderly above the age of sixty-five, are more likely to experience hospitalization caused by

severe RSV infections.[6] Having a preexisting medical condition also puts people at higher risk for contracting severe RSV infections that could potentially develop into RSV bronchiolitis or RSV pneumonia.[6] For instance individuals with chronic lung disease or congenital heart disease are at highest risk of developing severe forms of RSV infections that will possibly lead to hospitalizations and increased risk of death.[3] To provide another example, there is evidence of RSV infections persisting in people with chronic obstructive pulmonary disease (COPD), which leads to worsening of COPD symptoms such as wheezing and coughing, increased airway inflammation, and suppressed immune response in the lungs.[7] For most people with a pulmonary disease, an RSV infection could persist for prolonged periods of time and lead to permanent effects on their respiratory system that leave them susceptible to future comorbidities.

Infants and children are at particularly high risk for contracting RSV. It is estimated that almost all children are infected with RSV by the time they are two years of age, meaning that children and infants make up the majority of patients who fall victim to the virus.[8] Lung development continues until about the age of two, so young children suffering from severe RSV infections are at increased risk of experiencing impaired lung development which can lead to developing morbidities later in life.[1] Adding to this complication, children under the age of two who have experienced at least one RSV infection are vulnerable to reinfections in the future, which poses a challenge for both patients and medical professionals.[1] Due to the many physiological consequences of contracting early RSV, the development of an early prescribed RSV vaccine could provide intervention against long-term lung disease. A successful RSV vaccine would need to be safely administered to anyone, but especially young children, without causing adverse side effects. Independent of a vaccine, ribavirin and monoclonal antibody

therapy that will reduce the risks of future morbidities following early RSV infections are also being considered.[1]

Pathophysiology of RSV

The pathogenesis of RSV infections help to explain the different mechanisms that may contribute to the long-term effects of this virus. The virus is introduced to an individual via respiratory droplet from contact with saliva or mucus from an infected individual and incubates in the nasopharynx for two to eight days before traveling down to the pharynx and entering the conducting airways of the respiratory tract.[1] Once inside the conducting airways, RSV often targets the apical surfaces of ciliated epithelial cells lining the trachea and bronchi; it is suspected that the cilia aid in the spread of RSV infection to neighboring epithelial cells through the action of ciliary beating.[9] The virus has two surface proteins that make up its viral antigens.[10] These surface proteins are the G protein, which allows attachment between RSV and the host cell, and the F protein, which allows the plasma membrane of the virus to fuse with the host cell plasma membrane so that the viral antigens are released directly into the host cell **(Figure 1)**.[10]

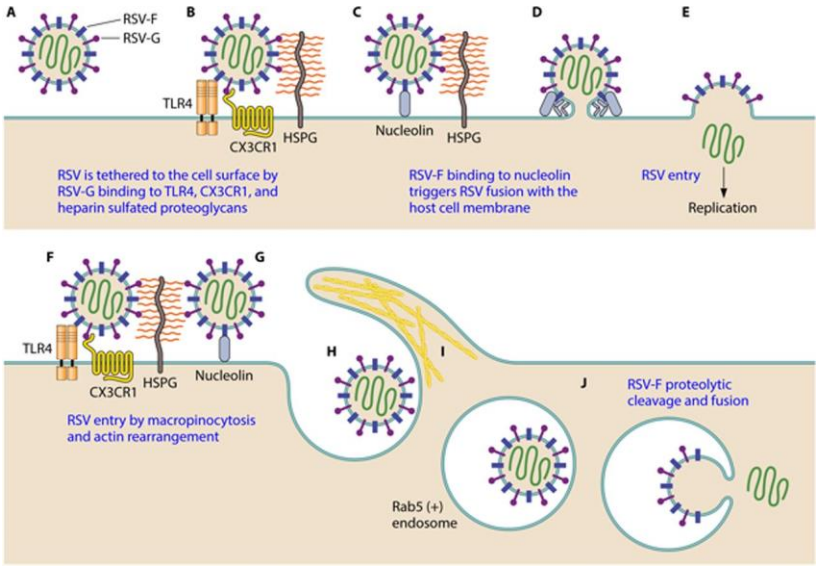


Figure 1 RSV Entry in Host Cells Two methods by which RSV empties its viral components into the host cell is shown above. The first method (top panel) shows the G surface protein binding to a transmembrane protein (TLR4) a G-protein coupled receptor (CXCR1), and a heparin sulfated proteoglycan (HSPG) which anchor the virus to the host cell **(A-B)**. The F surface protein then binds to a nucleolin receptor that allows RSV to fuse with the host cell's membrane **(C-D)**, resulting in cell entry **(E)**. The second method (bottom panel) contains similar cell binding steps **(F-G)**, however RSV is engulfed by the host cell **(H-J)**, and the F protein enables proteolytic cleavage of the surrounding endosome which allows the release of viral contents into the host cell **(J)**. The yellow rods in **I** represent actin filaments. Original figure from (Griffiths et al, 2016)

After the virus's contents are released into the host cell, intracellular replication of the virus can begin **(Figure 2)**.[1] RSV uses transcription and translation proteins from the host cell which transcribe its viral RNA strand into a new complementary strand and allows for host cell proteins to synthesize and replicate new viral RNA to either be packaged and released as new viral microbes or be continuously transcribed to generate more viral RNA strands.[10] RSV has an RNA-dependent replication cycle, it does not have an efficient proofreading mechanism, so RSV replication is prone to error.[11] As a result, the mutations that cause changes between the original genome and the newly replicated genome occur often, making it difficult to create a vaccine for a virus that has the potential to frequently mutate.[12]

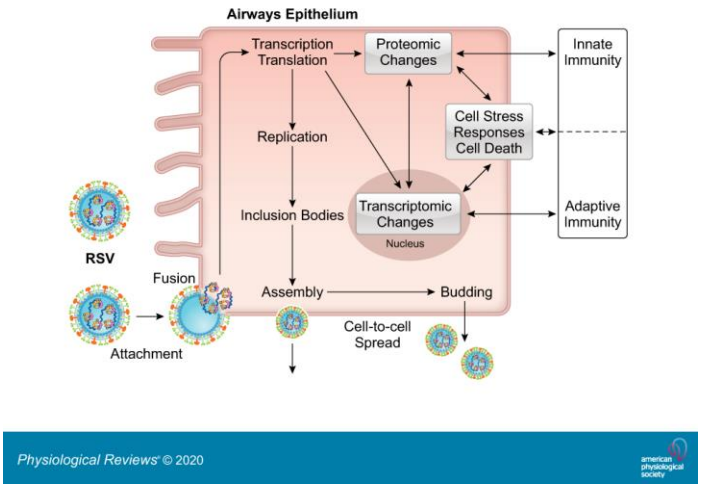


Figure 2 RSV RNA Replication in Host Cell RSV attaches and fuses with the host cell membrane, allowing entry of RSV. The RNA strand of RSV undergoes transcription and translation in the cytosol of the host cell with transcription and translation proteins provided by the host. The newly replicated RNA strand induces the formation of inclusion bodies which helps to protect the synthesis of viral RNA. The new viral RNA strand then assembles a plasma membrane and surface proteins before leaving the cell to infect neighboring cells. More than one RSV virus can leave the cell through a process called budding. Original figure from (Hu et al, 2020).

The invading virus does not go unnoticed for very long, however, due to the elaborate immune response to foreign microbes in the respiratory system **(Figure 3)**. One of the first actions of respiratory immunity begins with release of small signaling peptides (cytokines) and induction of an inflammatory response at the site of the RSV infection as part of the innate immune response.[9] During this time, the infected epithelium produces chemokines (cell-attracting cytokines) that aid in the inflammatory response and signal for certain immune cells, such as monocytes, macrophages, and neutrophils, to try to contain the viral infection.[9] Of the immune cells that take part in this early immune response, neutrophils provide one of the largest responses to RSV infection.[13] Neutrophils respond to the viral infection by phagocytosing the infected host cell.[13] Natural killer cells eventually show up to the scene to kill the infected host cell along with the virus and help slow the spread of infection, but their protective cytotoxic properties combined with their overproduction can lead to injury in the lung tissue.[14] As a result, natural killer cells that specifically target viruses like RSV are reduced during an infection while certain other natural killer cells regulate inflammation.[13]

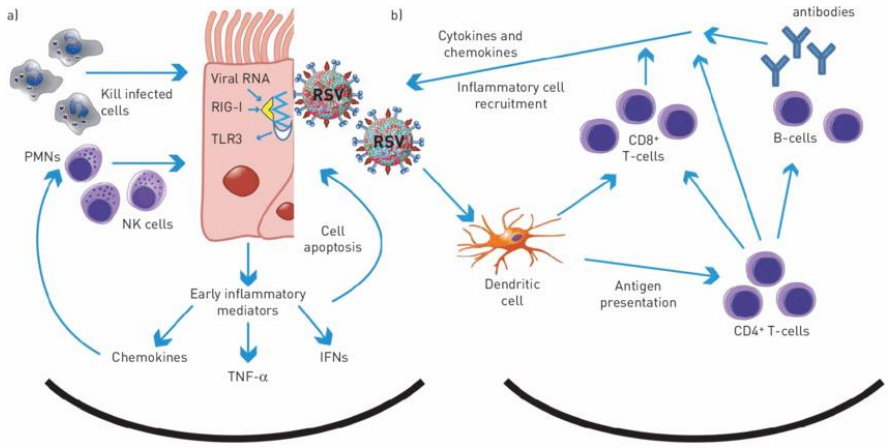


Figure 3 Innate and Adaptive Immune Responses to RSV **a)** Innate immune response to RNA infection. Viral RNA within the cytosol of the host cell is recognized by retinoic acid-inducible gene (RIG)-I-like receptors and toll-like receptors. These receptors activate early inflammatory mediators such as chemokines, interferons (IFNs), and tumor necrosis factor (TNF-α) which then recruit natural killer cells polymorphonuclear leukocytes (PMNs). The natural killer cells and PMNs can then kill the infected host cells. **b)** RSV can be processed by dendritic cells that present parts of the viral antigen to $CD8^+$ T cells (cytotoxic T cells) and $CD4^+$ T cells (T helper cells). CD4+ T cells then activate CD8+ T cells and B cells, which produce antibodies, to recruit inflammatory cells and further recruit innate immune response cytokines and chemokines. Original figure from (Rossi and Colin, 2014).

In regards to removing RSV from the lungs, adaptive immunity plays a critical role in destroying the virus and building up immunity in case of reinfection. Dendritic cells in the nasopharyngeal mucosa and in the conducting airways are another important contributor to the immune response during RSV infection.[13] Dendritic cells are the primary antigen-presenting cells that first detect foreign bodies like RSV; macrophages and monocytes can serve as secondary antigen-presenting cells within the lungs.[13] Macrophages phagocytose infected epithelial cells and present pieces of the virus on the surface protein MHC-II for T helper cells to recognize and signal for antibodies and cytotoxic T cells to kill the infected target cells.[13] Dendritic cells endocytose the viral antigen, break it down to collect vial peptides, and migrate to a lymph node to present a viral peptide on MHC-II proteins for helper T cells to recognize and on MHC-I proteins for cytotoxic T cells to recognize.[15] At the beginning of an RSV infection, cytotoxic T cells and helper T cells do not increase in number, but as the infection progresses, there is a significant increase in cytotoxic T cells, which are important for eliminating the virus.[13] There is also a moderate increase in helper T cells, which are important for activating both B cells that produce antibodies and cytotoxic T cells that eliminate infected host cells **(Figure 3)**.[13] With a stronger immune response available, the virus and infected host cells are eliminated through phagocytosis and apoptosis, and the infection can be managed so that the airway tissue can recover without permanent damage or "remodeling." [9]

RSV infection manifestations can differ among individuals. The severity of an infection varies from person to person and depends on factors, such as the patient's age, environmental exposures to allergens or air pollutants, any comorbidities that the patient has, and whether or not the patient has had a previous RSV infection, in which reinfection elicits a more efficient immune response.[10] If an individual has undergone an antibody response against RSV through

previous infections, then future infections are typically less severe. However, a first exposure to RSV precludes a previous RSV antibody response, so the patient will rely on innate immune events until adaptive immune cells has time to produce antibodies and cytotoxic T cells to eliminate the virus and infected host cells.[10] The location of an RSV infection plays an important role in severity, as well. Upper respiratory infections in the large conducting airways and bronchi caused by RSV tend to be less severe as it mostly presents cold-like symptoms and occurs in older children and adults while lower respiratory tract infections (LRTIs) that occur in bronchioles and alveoli tend to occur in infants, elderly patients, and immunocompromised patients and present more severe symptoms, such as difficulty breathing, cyanosis, and severe fever.[1] The severity of an RSV infection can be influenced by the type of strain, too, with an "A" type strain often showing stronger correlation to greater severity than a "B" type strain.[1]

While an RSV infection can lead to the development of illnesses like bronchiolitis and pneumonia, it can also cause damage to lung tissue, specifically for children under the age of two.[1] The immune response to an RSV infection leads to the sloughing off of dead ciliated epithelial cells in the large conducting airways, which exposes the underlying tissue to the lumen of the airway and leaves nerve fibers from the cough reflex more sensitive and susceptible to damage or pathogens.[10] In addition to the sloughing off of dead epithelial cells, there is increased mucus secretion and increased viscosity of the mucus, and the combination of dead epithelial cells and thick mucus that builds up in the lungs creates obstruction in the airways.[12] Infants are born with airways that are still developing and are smaller in diameter compared to an adult's, so any kind of obstruction caused by an RSV infection can cause severe difficulty in breathing and dangerously low oxygen levels in the blood.[12] Inflammation caused by an RSV infection also induces swelling, which constricts the small airways on top of the airway

obstruction caused by cell debris and mucus, making it harder for infants to breathe.[12] Therefore, an acute LRTI caused by RSV can be very dangerous for infants and children under the age of two since the underdeveloped airway is more susceptible to respiratory conditions such as wheezing and obstruction. Further, LRTI in infants can alter the development of the lung and lead to long-term asthma and/or chronic obstructive pulmonary disease.[9]

Medications and Treatment for RSV

When treating RSV infections, ribavirin is one of the most effective medications currently being administered to patients, and so far, it is the only licensed medication that has earned wide approval from medical experts.[12] Ribavirin is synthetic nucleoside analog that is used as an antiviral drug.[16] Ribavirin can be administered both orally and in aerosolized form.[17] The aerosolized form of the drug is recommended for younger at-risk populations like infants and toddlers.[17] Ribavirin inhibits the RNA strand of RSV from replicating in the host cell by capping the RNA strand or inhibiting its replication polymerase.[18] A number of clinical trials performed on RSV-infected patients have shown that using ribavirin as treatment for RSV infections can decrease viral shedding, shorten hospitalization time, and provide faster RSV clearance from the respiratory tract.[12] On top of reducing hospital stays for those affected with RSV, ribavirin is also capable of reducing the reliance on a ventilator in response to severe RSV infection responses.[19] Despite ribavirin's effectiveness in clinical settings, there are issues centered around the expensive costs for ribavirin, and there are debates on whether or not the sometimes limited beneficial outcomes make it worth the expense.[19] The lack of consensus on the mechanism of how ribavirin functions also raises concern.[17] Studies have shown that ribavirin exhibits significant improvements when treating severe RSV infections, but its prolonged use can be toxic.[20]

In addition to the broad-based approach exemplified by ribavirin, targeted biologics are being explored as potential treatments for RSV. These biologics include monoclonal antibody treatment directed at RSV. Of these antibody treatments, palivizumab is a popular one which can be described as an early antibody that plays a primary role in the prevention of RSV infection in the respiratory tract by helping the body build immunity to RSV prior to infection.[21] Since palivizumab is important for its preventative properties, it is often administered to high-risk children and premature infants who are at higher risk of acquiring severe RSV infection and are likely to suffer long-term consequences.[22] Another monoclonal antibody that shows promise in RSV prevention is motavizumab, which is derived from palivizumab.[21] Motavizumab results in greater prevention of RSV in high-risk children and greatly reduces the number of hospitalizations and lower respiratory tract infections experienced by children.[21] The promise of antibody therapy against RSV suggests that a usable vaccine for RSV, where patients make their own antibodies, may be a viable treatment to prevent RSV.

Use of neutrophils to regulate immune responses during an RSV infection is another potential treatment option.[8] By stimulating production of neutrophils, the immune system strengthens its ability to defend against RSV infections and prevents the spread of the virus.[8] Relying on neutrophils remains a concern, however, since they can cause tissue damage in the respiratory tract that may be harmful to individuals infected with RSV, especially infants.[8] Despite this, neutrophils are beneficial for directly combating viral antigens during infection, so finding ways to regulate neutrophils for antiviral activity and minimizing tissue damage could introduce new forms of RSV treatment.[8]

RSV treatments vary in efficacy and professional recommendation, but research on preventative strategies offer promising results. If RSV infections can be prevented altogether,

then the risk of developing future morbidities will be less concerning. Effectively preventing RSV infection can reduce lung damage that can manifest into chronic lung disease.

Asthma

Asthma is a chronic condition that primarily affects lung airflow through inflammation of the cells lining the airways.[23] With asthma, the airways become hypersensitive to particular stimuli that trigger inflammation of the cells lining the airways and cause these airways to constrict, resulting in obstructed airways and decreased airflow.[24] As inflammation occurs, excess mucus is produced and secreted into the airway, which only adds to the obstruction of airflow.[25] Airway remodeling is another contributing factor that characterizes asthma, and this process consists of increased protein deposition from the epithelial cells lining the airways as well as increased smooth muscle in the airways.[26] The severity of one's condition also differs from person to person such that some individuals may find that asthma rarely disrupts their daily lives while others are impacted on a larger scale and suffer from frequent asthma attacks.[27] Individuals who suffer from severe asthma are said to have uncontrolled asthma that can be attributed to factors such as first-time symptoms of asthma, ineffective inhaler technique, ineffective medication, or misdiagnosis of asthma for another condition.[28] Those who have less severe conditions and are better able to manage their asthma are considered to have well-controlled or partly controlled asthma.[28]

Asthma affects millions of people.[25] Both children and adults can be diagnosed with asthma, but studies show that there is a higher rate of diagnoses for asthma among children.[29] Some symptoms of asthma can include wheezing, coughing, tightness in chest, and experiencing shortness of breath or difficulty breathing.[30] These symptoms can persist throughout one's life,

or they could subside for several years until the airways experience agitation from environmental allergens or prolonged exposure to poor air quality, typically called an asthma "exacerbation" or an "asthma attack."[29] Asthma does not have a cure, but the symptoms can be treated. Airflow can be maintained with proper treatment such as using an inhaler, a device that allows people to take aerosolized medications like beta adrenergic agonists and corticosteroids or it can be maintained by avoiding environmental factors that may trigger asthma exacerbations/attacks, such as allergens and pollutants.[23]

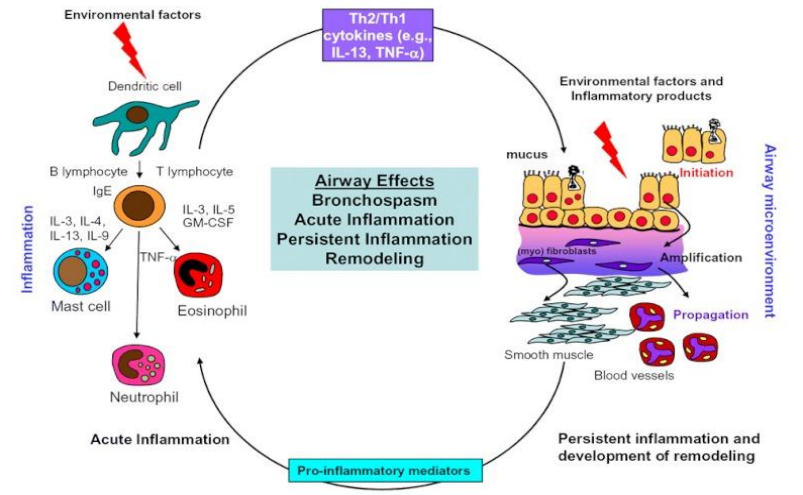
In situations where efficient treatment is not easily attainable or treatment is not used on a regular basis, problems can arise in daily life and activities. Asthma can be associated with frequent visits to hospital emergency centers and unanticipated visits to primary care providers depending on the severity of one's condition.[31] These visits can be expensive and alarming to the family of the patient if the chronic condition leaves the patient in poor health.[31] In the cases for children and adolescents, frequent visits to the hospital due to asthma attacks can result in increased school absences, less participation in school activities and extracurriculars, and decreased school performance.[31] On the other hand, adults who experience complications with their asthma may need to take days off from work and neglect other responsibilities until they can manage their asthma well enough to function. The enormous health and financial burden of asthma worldwide and has spurred significant research effort to better understand mechanisms of the disease and novel avenues for treatment.

Pathophysiology of Asthma

The general mechanism of asthma entails that allergens or pathogens can trigger an immune response in the airways that induces constriction of the smooth muscles surrounding the airways.[29] The sensitivity of one's airways varies from person to person, but the immune

response is consistent between asthma patients by starting with inflammation. Inflammation in asthma begins similarly to how it would during an RSV infection where cytokines and chemokines are produced by airway epithelial and other cells, and these cytokines trigger the proper immune response through cellular signaling.[32] Among the cytokines activated during an asthma attack, interleukin-5 (IL-5) plays a crucial role in the growth and differentiation of eosinophils which are important for determining whether individuals develop eosinophilic asthma or noneosinophilic asthma.[33]

Eosinophilic asthma is more common among the two types and arises from high eosinophilic counts in the blood and airway in response to foreign agents in the respiratory system.[33] The large number of eosinophils recruited by IL-5 during the innate immune response lead to expansive eosinophilic inflammation.[33] High levels of eosinophils in the airways then lead to hyperresponsiveness in the airways that contribute to narrowed airways and heightened inflammation.[33] Since IL-5 is a major contributor to eosinophilic asthma, it has become a focal point for research in preventing the pathogenesis of eosinophilic asthma entirely.[33] With noneosinophilic asthma there is a low eosinophil count in the airways and blood but higher counts of neutrophils and inflammatory granulocytes, or leukocytes containing granules.[34] The inflammatory response for noneosinophilic asthma is similar to the inflammatory response seen in eosinophilic asthma besides the differences in how many cytokines are activated and which leukocytes are activated during the immune response.[34]



Key: GM-CSF, granulocyte-macrophage colony-stimulating factor; IgE, immunoglobulin E; IL-3, interleukin 3 (and similar); TNF-α, tumor necrosis factor-alpha

Figure 4 Adaptive Immune Response in Asthma The cycle above shows environmental factors activating dendritic cells in the nasopharynx and/or large airways. The dendritic cell presents the foreign antigen for T cells and B cells to recognize. These cells activate immunoglobulin E (IgE) production which recruits several inflammatory mediators. The interleukins IL-3, IL-4, IL-13, and IL-9 recruit mast cells; tumor necrosis factor alpha (TNF-α) recruits neutrophils; and IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) recruit eosinophils. T helper cells 2 and 1 cause an amplification of the inflammatory response which leads to increased mucus secretion, propagation of smooth muscle cells (airway remodeling), and persistent inflammation via pro-inflammatory mediators Airway effects include bronchospasm (bronchoconstriction), acute inflammation, persistent inflammation and airway remodeling . Original figure from (NIH, 2007).

In a typical immune response, neutrophils and monocytes phagocytose foreign bodies like allergens and pathogens that are causing a flare up in asthma patients **(Figure 4)**.[32] Macrophages and dendritic cells then play important roles as antigen-presenting cells, which results in the amplification of the inflammatory response via adaptive immunity.[35] The adaptative immune response is initiated with B cells producing antibodies and cytotoxic T cells aiding in the elimination of foreign microbes.[32]

Alongside airway inflammation, asthma is characterized by bronchoconstriction, or narrowing of the airways.[36] One way for bronchoconstriction to occur is when airway smooth muscles contract and force the narrow airways to fold inward.[36] This can cause asthma patients to experience shortness of breath, coughing, and wheezing.[36] The epithelial cells lining the airways undergo infolding, further constricting the airway and leading to mechanical stress that causes stretching and compression that damages the airways.[36] Apart from allergens and pollutants, bronchoconstriction can also occur through physical stress created by exercise which

forces airway smooth muscle cells to contract and narrow the airways.[35] While initial changes can be acute and reversible, chronic affects lead to the bronchioles undergoing airway remodeling due to frequent bronchoconstriction, and this promotes more permanent increased wall thickness of the bronchioles and increased thickness of the airway smooth muscle.[37] The overall effect of this airway remodeling is a decrease in total lung compliance and an increase in total lung resistance, leading to increased breathing complications during an asthma attack.[37]

Among airway inflammation and bronchoconstriction during an asthma attack, columnar epithelial cells and mucus-secreting cells secrete excess mucus into the airways as a defensive property against pathogens or foreign microbes.[32] Hypersecretion of mucus causes further obstruction of the airways **(Figure 5)**.[32] These variables combined contribute to breathing complications in asthma and lead to several different areas to target for asthma relief. Optimal treatments that can reduce airway inflammation, bronchoconstriction, and airway obstruction could all be effective in reducing asthma burden. While there are treatments that are capable of alleviating some of these symptoms, treatment that

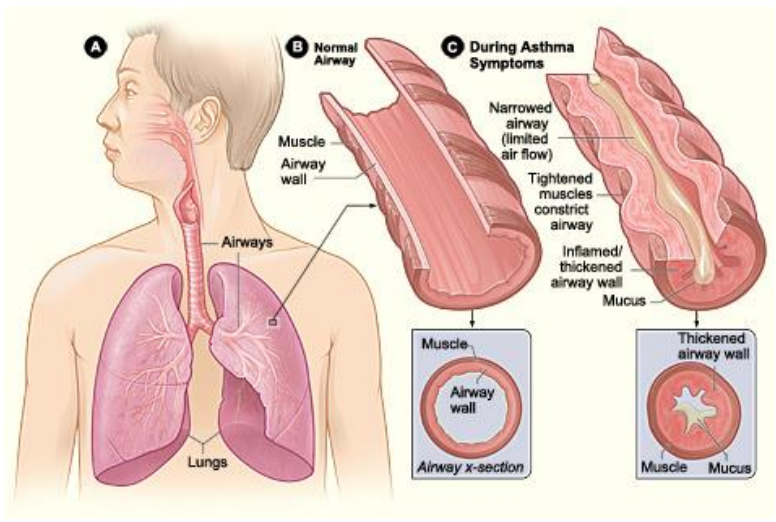


Figure 5 Visual of Asthmatic Conditions (A) General anatomy of the lungs, the conducting airways, and the bronchi/bronchioles. (B) Normal airway – the dark strips surrounding the airway wall are smooth muscle cells. The cross-section of the airway wall and airway smooth muscle is thin, and the diameter of the lumen is large in normal airways. (C) shows the airways during asthma symptoms. The airway wall is thickened and inflamed, the airway smooth muscle is constricting the airway wall and narrowing the lumen of the airway, and excess mucus is causing airway obstruction. In the cross-sectional view, the diameter of the lumen is smaller, and the airways walls are thicker. Original figure from (Allergy and Air, 2014).

address all three variables and/or treatments that effect upstream events that lead to the observed symptoms are not yet available.

Medications and Treatments for Asthma

Since asthma cannot be cured, there are several options for preventing and relieving symptoms. Inhalers are largely associated with asthma, and three types of inhalers can be used by patients including the dry powder inhaler, the soft mist inhaler, and the most commonly used pressurized metered-dose inhalers.[38] A meta-analysis that reviewed the effectiveness between different types of inhalers found that there is no major difference in effectiveness among inhaler types; outcomes largely depends on the patient's compatibility with a particular inhaler.[39] Studies did find that meter-dosed inhalers are the most cost-effective, which could explain their popular use among asthma patients.[39]

The use of bronchodilators is the most common asthma treatment medication.[40] They are used to relax constricted smooth muscle surrounding the airways and result in a more "open" airways to improve airflow and breathing.[40] There are generally two forms of bronchodilators. The short-acting bronchodilators relieve symptoms at a fast rate and are used for treating severe asthma symptoms; and the long-acting bronchodilator keep the airways open for prolonged periods of time and reduce the chances of an asthma attack.[41] Short-acting and long-acting bronchodilators both fit into a category of beta 2-agonist bronchodilators.[41] Short-acting beta 2-agonists include albuterol possibly combined with ipratropium bromide and levalbuterol, while the long-acting beta 2-agonists include salmeterol, formoterol, and combined medications.[41] There is wide variability among which beta 2-agonists are the most effective in reducing asthma symptoms; for example, one study that reported greater lung improvement with salbutamol while others reported greater lung improvement with ipratropium.[42] Anticholinergics are another type

of bronchodilator used by asthma patients, and they are often paired with beta 2-agonists to increase the effect of bronchodilation.[43] However, anticholinergics on their own do not demonstrate significant improvement in asthma symptoms the way beta 2-agonists do.[43]

Inhaled corticosteroids are another asthma treatment paired with inhaler use.[40] Inhaled corticosteroids are an anti-inflammatory medication that reduces airway inflammation and constriction of the airways to allow for efficient breathing.[40] The use of inhaled corticosteroids are an indicator of the severity of one's asthma condition by which an individual likely has heightened airway sensitivity and persisting symptoms.[44] Corticosteroids are commonly used in combination with bronchodilators within an inhaler, so researchers study the combined effects of these asthma medications as well as their effectiveness by themselves.[40] For example, Chauhan et al conducted a study comparing the effects of inhaled corticosteroids to the combination of long-acting beta 2-agonists and inhaled corticosteroids in children with chronic asthma.[45] They found that the combination of the medications presented significant improvement in lung function.

Asthma patients have more treatment options aside from inhalers. Some individuals with asthma, specifically infants or young children, use a device called a nebulizer, which turns liquid medication into a mist so that it can be inhaled through a face mask or mouthpiece without complication.[46] The medication used in a nebulizer is normally a bronchodilator like albuterol, and this helps with airway hypersensitivity and difficulty breathing.[46] Albuterol treatment with a nebulizer are highly recommended for acute asthma symptoms and keep symptoms from worsening.[46] A major concern with albuterol treatment is that inappropriate dosages can be administered at home which may lead to other morbidities and possibly death in severe cases.[46] In cases where asthma is triggered by allergens like pollen or dust, allergy shots are a useful for

relieving symptoms and building up tolerance to whichever allergen irritates the patient's respiratory system.[47] Also called allergen immunotherapy, allergy shots can be administered to both adults and children over the age of five.[47] They need to be administered every couple of weeks for twelve months in order to build up immune tolerance against allergens.[40] The effects of allergen immunotherapy depend on the length of dosage treatment and can have lasting effects for several months.[47] Overall, the type of medication that people use to treat their asthma depends on a variety of factors, including compatibility and responsiveness to a medication, costs of medication, severity of one's asthma, and a patient's age and overall health.

Research Findings

RSV Association with Asthma

Over the past several decades there continues to be new findings and supportive evidence for a causal link between RSV and asthma. To begin with, the age at which an individual contracts RSV and the severity of the infection are important contributors to long-term effects of having the virus. Due to high infection rates among infants and children, a vast amount of studies are centered around young children with premature infants and high-risk children being two of the most widely studied populations.[48] Given this information, Meijas et al began studying full-term infants who have contracted RSV within the first year of life and monitored any reports of asthma or wheezing development over a course of sixteen years.[48] Among these findings, Meijas and his colleagues found that having a serious RSV infection within the first year of life resulted in higher risk of developing asthma by the age of five.[48] Their findings suggest that early prevention of RSV within the first year of life could in fact reduce the risk of asthma development later in life.[48] In another study, Stockman et al notes that the rates of RSV hospitalization in children and infants decreases as age increases, indicating that age is a critical

factor in the severity of RSV infection.[49] Administering RSV immunoprophylaxis to high-risk infants and children also demonstrated a reduction in RSV hospitalization rates by one-third compared to the rates shown in the control group.[49]

Epidemiological studies have focused on the history of RSV hospitalization and future development of asthma. In one study, Jonathon Coutts and his colleagues took information from a national population database in Scotland and found that RSV hospitalization (RSVH) within the first two years of life is associated with higher incidences of asthma hospitalizations in following years.[50] Their results indicate that the likelihood of developing severe asthma is correlated with the severity of an RSV infection during early childhood.[50] They found that twice the amount of asthma hospitalizations occurred among children who had a history of RSVH in the past compared to the control group that did not have a history of RSVH.[50] Coutts et al also refers to a number of studies with evidence of RSV hospitalizations during infancy being associated with asthma development during later childhood.[50] Across these studies, researchers discovered that the asthma rate in children is about 1.5 to 4.5 times higher for children with a history of RSV-induced hospitalization compared to children in the control group who did have asthma but were never hospitalized from an RSV infection.[50] Adding to these findings, Homaira et al conducted a population-based study that measured the rate of asthma hospitalizations between children who suffered RSVH after the first six months of life and children who suffered RSVH within the first six months of life.[31] They discovered a 2- to 7-fold increase of asthma hospitalizations among children who suffered RSVH after the first six months of life, which implies that the age at which an individual is hospitalized from RSV is an important factor in determining future asthma development and asthma severity.[31]

While it is difficult to pinpoint the exact causal link between RSV and subsequent asthma development, there are plenty of theories on which biological factors contribute to this association. Most studies are beginning to identify certain biomarkers of both asthma and RSV infection to see whether if the markers present during an RSV infection are predictors of future asthma development.[51] One significant biomarker is the cytokine Interleukin-33 (IL-33), which is presumed to be associated with asthma genesis.[12] In a study with mice, RSV gives rise to IL-33 gene expression within neonate lungs but not within the lungs of adult mice.[12] From this study, researchers concluded that increased levels of IL-33 resulting from RSV infections within neonate mice can determine the severity of asthma within adult mice.[12] Griffith et al reasoned that this event occurred because high levels of IL-33 expression in the lungs correspond to severe asthmatic conditions.[12] Another pro-inflammatory cytokine that plays a significant role in asthma genesis is Interleukin-13 (IL-13).[12] IL-13 can be responsible for the increased levels of IL-33 presented in the mice study mentioned before, which serves as another possible connection between RSV infection and subsequent asthma development.[12] IL-13 is expressed by type 2 innate lymphoid cells (ILC2s) and ca lead to mucus production and airway reactivity that leads to asthma exacerbations.[52] Another study using mice sought to measure the production of IL-13 in the lungs following an RSV infection and found that four days after the infection resulted in increased concentrations of IL-13 and increased concentrations of IL-13-producing ILC2s in the lungs.[52]

Saravia et al set out to determine the age-specific response of IL-33 which could be used to understand the immunopathophysiology of RSV-induced morbidities like asthma.[53] Saravia and her colleagues recognized that T helper 2 cell immunity (Th2 immunity) was closely associated with ICL2 and IL-33 activity during an RSV infection.[53] It turned out that infants

rely heavily on Th2 adaptive immunity and type-2 immune responses, and these immune responses would increase the release of IL-33 and IL-13 during an RSV infection.[53] Their results demonstrated that IL-33 levels and IL-13 levels in neonate mice were significantly higher than the levels found in the lungs of the adult mice, indicating that age of initial infection is crucial to the development of these immune responses.[53] In addition to these findings, Saravia et al pointed out an increased ICL2 expression in the lungs in response to increased IL-33 levels in the lungs, which would provide insight on how early RSV infection can lead to other morbidities like asthma.[53]

Since IL-13 and IL-33 are both associated with ILC2s, it would make sense that ILC2s also take part in the development of asthma. In fact, activated ILC2s contribute directly and indirectly to a number of different asthma symptoms, including smooth muscle contraction, overproduction of mucus, and airway hyperactivity.[28] The mechanism that promotes IL-13 production in the lungs begins with the stimulation of IL-33 receptors which can be found along the surface of ICL2s.[28] ILC2s then induce airway inflammation mediated by IL-13 production and signals the activation of the innate immune response.[28] In summary, the early stage of an RSV infection causes IL-13 levels to rise to promote inflammation.[28] This inflammatory response leads to the recruitment of IL-33 and increased levels of IL-33 that activate the IL-33 receptors on ILC2s.[28] Once these receptors are stimulated, ILC2s produce more IL-13 cytokines that continue the cycle and strengthens the innate immune response.[28] Overall, the combined results of these studies have identified biomarkers that are present in both asthma genesis and the immune response against RSV infections, suggesting a few biological links between RSV and subsequent asthma development.

Potential for RSV Vaccine as Asthma Therapy

While researchers continue their efforts to pinpoint an exact causal link between respiratory syncytial infection and subsequent asthma, other studies are searching for preventative measures against RSV infections altogether. Finding a suitable vaccine for groups that are the most susceptible to severe RSV infections is one of the main goals for numerous studies on this topic. Although, a proper vaccine that demonstrates effective and safe preventative properties against the virus is yet to be discovered. While some studies declare that a successful RSV vaccine will not be available for several years, other studies are discovering promising results for a future vaccine.[54] One such testing involves a live-attenuated vaccine that deletes a coding sequence in the RSV genome that allows the ability for G-protein attachment.[55] The significance of deleting this coding sequence is that in the absence of the G-protein the efficacy of RSV entry and RSV replication within host cells is reduced, which gives the immune response enough time to identify and destroy the virus.[55] In this study, the live-attenuated vaccine, called RSV delta-G, appears to be safe among human adults with adverse side effects being mild to moderately severe.[55] These effects were either short-lasting or resolved without worsening effects.[55] With promising results regarding the safety of this novel vaccine, there are plans to someday test the effects of this vaccine on children, who are one of the target populations for severe RSV infections.[55] Support for live-attenuated vaccines for infants older than six months of age to children younger than the age of two relies on the mimicry of a natural case of RSV infection without risk of enhanced RSV disease (ERD).[54] ERD is a noticeable side effect when children are treated with formalin-inactivated RSV vaccines.[54]

The formalin-inactivated RSV vaccine (FI-RSV) was one of the first RSV vaccines that went into testing over fifty years ago.[56] Newer versions of FI-RSV have yet to be administered in

trials with humans until researchers can find a way to eliminate the risk of developing ERD in human participants.[56] The primary issue was that vaccinated infants who had never been exposed to RSV suffered from ERD while older children and adults who have had previous exposure to RSV were unaffected.[54] Since then, effects of newly developed FI-RSV vaccines have been observed in animal models and human *in vitro* models, however, safe clinical trials are required to fully capture the human immune response in a natural setting.[56]

Another approach to vaccine development is the focus on specific RSV F and G proteins. Such a vaccine that can either block the F protein from fusing with the host cell membrane and thus prevent the virus from unloading its contents into the cells or; alter the G protein in such a way that it impedes its role in anchoring the virus to host cells.[56] While researchers struggle to find a safe and effective vaccine for infants and young children, another line of research is looking toward administering vaccines to pregnant women so that the expecting mother builds up antibodies against RSV.[54] The mother's antibodies can then be transferred through the placenta to the fetus so that the infant is already born with some immunity to RSV.[54] The main concern with maternal vaccinations is preventing any adverse effects regarding fetal development, such as congenital abnormalities, or possibilities of miscarriage.[54]

At this time, there is no vaccine that both has made a significant impact in preventing RSV infection and can be administered safely to people of all ages. One limitation for those researching a vaccine is the fact that they use animal models, such as mice, rats, or pigs, which may provide useful information but do not imitate the same immune responses as humans when undergoing vaccination trials.[56] Other concerns with finding an RSV vaccine is whether the vaccine will be cost-effective and whether it can be utilized by anyone, especially the target populations who are at higher risk of infection.[54] With reinfection as a possibility and the

knowledge that RSV can mutate, an RSV vaccine may need to resemble a flu shot such that it can be administered annually. For now, preventative strategies are being used to keep RSV infections in young children and infants at a minimum while reducing possibilities of subsequent asthma development.

Potential for RSV Prevention as Asthma Therapy

Researchers continue to study different preventative measures and treatments for RSV infection which come with varying results. Currently, ribavirin is the only RSV treatment that has gained widespread approval among the medical field, but its efficacy varies widely across studies.[17] Ribavirin is associated with reduced risk of RSV-induced mortality and viral load in RSV patients.[20] The antiviral drug presents the best results when treating for severe RSV symptoms, and this may contribute to the inconsistent results presented by the drug.[20] Both the oral and inhaled versions of ribavirin have been proven to be safe and well-tolerated for most patients of all ages, but inhaled ribavirin is more expensive despite its recommended use for infants and toddlers.[57] Since ribavirin is too inconsistent in its treatment of RSV, researchers are choosing to focus on RSV prevention via RSV immunoprophylaxis instead.[16]

Palivizumab, the most popular type of RSV immunoprohylaxis for high-risk populations, is a passive RSV antibody called RSV immune globulin G that is administered by injections.[17] This monoclonal antibody treatment is tolerated well among most populations and can even reduce the risk of hospitalizations for high-risk infants and children by about seventy-two percent.[58] High-risk infants including preterm infants and infants with chronic lung disease or congenital heart disease are recommended to take palivizumab.[16] Palivizumab is administered to this population monthly during RSV season to ensure RSV prevention.[16] However, the high

costs of using palivizumab often deters parents from giving their infants this preventative treatment, which contributes to the high rates of RSV infection among this population.[16] Interestingly, a recent study regarding RSV prophylaxis has presented another monoclonal antibody similar to palivizumab called Nirsevimab.[17] The results of this study found that the use of Nirsevimab decreased the rates of LRTIs and hospitalizations caused by RSV infections among infants.[17] Nirsevimab also exhibited no adverse effects on the participants during testing trials, making it a promising RSV prophylaxis for high-risk populations.[17]

There is no definitive RSV treatment that provides optimal results in preventing asthma, but studies show that RSV prevention might be the best option for asthma prevention. While the idea of an RSV vaccine is promising, at this time. RSV immunoprophylaxis appears to have the best chances of minimizing the association between RSV and asthma. The next section of this review will discuss plans for future research.

DISCUSSION

Without a vaccine for RSV nor a cure for asthma, both conditions pose a problem for populations all over the world, harming some groups more than others. At the same time, researchers continue to search for the causal relationship behind early RSV infection and later asthma development with the goal of efficiently preventing asthma exacerbations caused by RSV infections. Researchers are focusing their efforts on the downstream effects of the human immune response against RSV infections and comparing these findings to the immune responses observed in individuals with asthma.

Comparing the similarities between the inflammatory responses following RSV infection and asthma occurrence provides some overlapping changes that might provide early childhood intervention. Specifically, cytokines that are active during the inflammatory response caused by

RSV may enhance the inflammatory response that stimulates asthma pathogenesis. Targeting the mediators of the innate immunity inflammatory response could be a major step in preventing subsequent asthma development following early RSV infection. Identifying these causal mechanisms would make it easier for pharmaceutical research to design medications and treatments that can both treat RSV infections effectively and regulate the patient's immune response so that the airways experience less damage and become less hypersensitive. One issue that comes up is whether research needs to consider the downstream signaling effects of the inflammatory response. Since cytokines are important for the innate immune response, it is critical that RSV treatments and prevention strategies do not completely eliminate these downstream signaling events in order to allow the immune system to function properly against an RSV infection. Therefore, downregulating or inhibiting the interleukins mentioned in this literature review would require careful consideration of their downstream effects. Another option would be to try to regulate only one component of a downstream signaling event to reduce persistent inflammation caused by infection, but this goal still comes with numerous complications.

As for asthma prevention, turning IL-5 into a focal point for research might prove useful in preventing asthma pathogenesis. As stated above, IL-5 contributes to inflammation during an asthma attack in part by increasing the numbers of eosinophils in the lungs.[33] Currently, there is no effective way to downregulate eosinophilic inflammation through regulation of IL-5, but it could be important to monitor young children's eosinophil counts each time they contract an RSV infection. This would allow doctors to observe any changes significant changes in the children's eosinophil count that resemble early signs of asthma genesis.

Like IL-13 and IL-33, IL-5 is released from ILC2, and this information provides yet another probable link between the causal association between RSV and subsequent development of asthma.[33] Just as severe RSV infections lead to increased concentrations of ILC2 followed by increased concentrations of IL-33 and IL-13, RSV infections might also lead to increased concentrations of IL-5 that bring rise to future asthma development. Therefore, it may be favorable to target one of the upstream effectors that produces IL-5, such as ILC2s, mast cells, T2h cells, or eosinophils themselves.[59] Downregulating the activity of these cells so that less IL-5 is produced and less eosinophils are recruited would greatly decrease asthma exacerbations caused by excessive inflammation. In fact, anti-IL-5 therapies are under development to target IL-5 and IL-5 receptors in hopes of decreasing eosinophilic inflammation and reducing asthma severity.[60] One type of anti-IL-5 therapy is mepolizumab which has shown significant efficacy in reducing asthma exacerbations and controlling asthma symptoms.[60] Despite whether or not RSV is a contributor anti-IL-5 therapies provide good reason for reducing IL-5 signaling events and protecting high-risk populations from developing asthma. If it is possible to control and prevent asthma through these therapies, then asthma prevention through targeting IL-5 and its upstream effectors would be a significant breakthrough among the medical community.

For now, the seemingly best way to slow the rates of asthma development in young patients is to try to prevent RSV infections altogether. RSV medications such as palivizumab and motavizumab are currently providing favorable outcomes in preventing RSV infection for patients, and they continue to be recommended by several studies. Currently, the use of monoclonal antibodies appears to be the most useful technique for preventing RSV as it allows at-risk populations to develop stronger immunity against the virus before RSV season begins. The goal for preventative RSV medication should be to improve the efficacy and delivery of the

medication to young patients while also making it affordable for all patients. Broadcasting public announcements on television or hanging informational posters at bus location could do plenty in advising parents on how to prevent RSV infection in their children during the winter months, in which RSV is more prevalent among target populations.

With researchers still motivated to discover a functional and safe vaccine for RSV, the crucial search for a COVID-19 vaccine may provide another path for the development of an RSV vaccine. Given that the COVID-19 pandemic has become a worldwide issue, there will most likely be a vaccine for the coronavirus before a vaccine for RSV is discovered. Finding a COVID-19 vaccine could be the solution needed to guide the development of a successful RSV vaccine. In particular, the focus on messenger RNA based vaccines may provide a template for development of similar vaccines for RSV. A particular concern would be if the COVID-19 vaccine could only be administered to older age groups, so researchers would have to adapt an RSV vaccine to patients of all ages, especially for infants and young children. Nevertheless, any lead on how researchers can develop a safe and functional RSV vaccine would be a step in the right direction.

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