REVIEW

New insights into the molecular mechanisms of Respiratory Syncytial Virus (RSV) disease

Giovanni **Piedimonte**

Correspondence to:
gpiedimonte@tulane.edu/ORCID: https://orcid.org/0000-0003-1640-1928

Doi
10.56164/PediatrRespirJ.2022.04

Departments of Pediatrics, Biochemistry & Molecular Biology, Tulane School of Medicine, Office for Research of Tulane University, New Orleans, LA, USA

ABSTRACT

Respiratory syncytial virus (RSV) infection is the most common respiratory infection in children and the primary cause of hospital admission in infancy. Also, multiple prospective epidemiologic studies have shown that early infection with this virus is a significant risk factor for wheezing and asthma in childhood and adolescence, even in subjects without atopic predisposition. Indeed, following RSV infection, the release of local pro-inflammatory molecules, the dysfunction of neural pathways, and the compromised epithelial integrity can lead to persistent hyperreactivity and inflammation that manifest clinically with recurrent episodes of airway obstruction. While most research work has been focusing on immune and inflammatory mechanisms, recent evidence has shown changes in the molecular structure of the epithelial and muscular airway cells that can drive airway dysfunction independently from canonic immune-inflammatory mechanisms and pathways. This article summarizes the most recent studies on some of the novel molecular mechanisms involved in the pathophysiology of RSV infection and the consequent predisposition to chronic airway dysfunction and asthma development, with some closing considerations on current and future treatment strategies.

IMPACT STATEMENT

RSV remains the most common respiratory pathogen in infants and young children and the most common cause of hospitalization in early life. Yet, no safe and effective therapy is available. A better understanding of the molecular pathophysiologic mechanisms outlined in this article might open the way to new preventative and therapeutic strategies able to reduce the morbidity and mortality caused by this virus.

KEY WORDS
Asthma; bronchiolitis; beta-adrenergic receptors; transient receptor potential cation channel subfamily V member 1 (TRPV1); neuropeptides; neurotrophins; neurogenic inflammation.

INTRODUCTION

Respiratory syncytial virus (RSV) is the most common respiratory pathogen in infants and young children worldwide (1). Prospective epidemiologic studies support

a strong association between RSV infection and acute pulmonary morbidity, such as lower respiratory tract infections (LRTIs), during infancy and long-term morbidity during childhood, such as airway hyperresponsiveness, recurrent wheezing, and asthma (2). The latter is believed to be closely related to a preexistent atopic predisposition with a T helper (Th)2-biased phenotype. This hypothesis implies the RSV- induced potentiation of T-cell responses to inhalant allergens mediated by local Th2 cytokines synthesis, eosinophils recruitment at inflammatory sites, and Th2-polarized RSV-specific immunological memory, which, following reinfection, leads to dense infiltrates of effector cells secreting interleukin (IL)-4, IL-5, IL-13, and other Th2-type cytokines and chemokines (3).

While the role of RSV as an independent factor favoring atopic asthma is widely recognized, its impact on the onset of non-atopic asthma, mediated via non-canonical pathways, has been more controversial. However, growing scientific evidence suggests that RSV-mediated persistent inflammation and airway hyperreactivity can result from alterations of the neural pathways and molecular changes in airway substructures that can occur in parallel and at different times of local and systemic immune responses (4). In the case of non-atopic asthma, such changes appear reversible after adolescence, suggesting a transient respiratory dysfunction rather than the progressive and irreversible damage commonly featuring atopic asthma (5). This review summarizes the most recent evidence about the molecular mechanisms involved in airway dysfunction and non-allergic asthma development following early infection with RSV.

RSV EFFECTS ON THE AIRWAY SUBSTRUCTURES

Epithelium

RSV enters the body primarily by direct contact with the nasal or conjunctival mucosa and then spreads from the upper respiratory tract to the lower airways, mainly targeting ciliated cells. In response to RSV infection, several changes occur in the airway epithelium, such as synthesis and release of inflammatory cytokines and chemokines that modulate the recruitment of inflammatory cells from the bloodstream into infected tissues and whose respiratory secretions concentrations usually correlate with infection severity. Concomitantly, the viral NS2 protein promotes epithelial cell shedding, which accelerates the clearance of virus-infected cells but, on the other hand, allows the accumulation of cell debris and mucus secretions that form myriads of plugs obstructing the distal airways (6).

Basal epithelial cells of the bronchial epithelium, intraepithelial dendritic cells (DCs), and alveolar type-I cells can also be infected by the virus (7) and release pro-inflammatory cytokines such as TNF-α, IL-33, and thymic stromal lymphopoietin (TSLP), which, in turn, promote Th2- type inflammatory responses and the recruitment of neutrophils and eosinophils (8). In neutrophils, RSV infection induces the expression of specific activation markers, such as CD11b, CD18, and CD54, and causes the release of neutrophil elastase. Moreover, neutrophil apoptosis and neutrophil extracellular trap (NET) are active during infection, and the peak of these activities coincides with maximum viral load and clinical severity (9). In eosinophils, RSV induces high levels of leukotriene C4, eosinophil-derived neurotoxin (EDN), and eosinophil cationic protein (ECP). The early detection of such inflammatory mediators in the respiratory secretions of patients with RSV bronchiolitis supports their role during the acute phase of infection (10).

Recently, the transient receptor potential vanilloid type (TRPV) family of non-selective cation channels has gained substantial interest from pulmonary scientists, especially $TRPV_1$ and $TRPV_4$ that are prominently expressed in the respiratory tract (11). Originally these channels were believed to be exclusively associated with nociceptive peripheral nerve fibers, where they trigger the release of sensory neuropeptides like the calcitonin gene-related peptide (CGRP) and tachykinins. Hence, their activation in response to chemical and physical irritants leads to “neurogenic” inflammatory reactions featuring bronchoconstriction, inflammatory cell chemotaxis, and mucosal edema (12). Surprisingly, the same channels were later found in a variety of mammalian tissues, including lung (bronchial epithelial and alveolar cells, smooth muscle), central nervous system, salivary and sweat glands, inner ear, heart, kidney, intestine, skin, endothelium, and fat tissue (13). Considering the mode of activation and the functions, it appears reasonable that prolonged and intense stimulation of $TRPV_1$ and $TRPV_4$ could play a crucial role in the pathogenesis of obstructive airway diseases, such

as asthma, bronchiolitis, and COPD. Also, because $TRPV_1$ and $TRPV_4$ are both involved into host-pathogen interactions including the binding, entry, and replication of viruses, these molecules might modulate viral-mediated airway damage. In this context, we first reported in an *in vivo* rodent model that RSV potentiates $TRPV_1$-mediated neurogenic inflammation (14). Similarly, another group reported upregulation *in vitro* of $TRPV_1$ expression 12 hours post-RSV infection. Interestingly, this effect was independent of virus replication, as virus-induced soluble factors were sufficient to increase channel expression. Moreover, capsazepine treatment inhibited virus-induced upregulation of $TRPV_1$, suggesting that these channels have an important role in virus-induced airway disease and cough, and hold promise as druggable targets for therapy (15). More recently, the pro-inflammatory functions of $TRPV_1$ were confirmed in human bronchial epithelium from children with asthma, both at baseline and after RSV infection (16). As illustrated in **Figure 1**, our studies showed for the first time that the lower airways epithelium from asthmatic children displays elevated basal and RSV-induced TRPV1 expression compared to non-asthmatic controls. This increase was measured both in total as well as plasma membrane bound $TRPV_1$. We also showed that RSV-mediated increased TRPV1 expression and activation is a consequence of increased signaling through the NGF-TrkA axis. Finally, we showed that TRPV1- mediated increase in $[Ca^{2+}]_i$ can originate from either the extracellular pool or intracellular storage sites within the endoplasmic reticulum, depending on asthma status and RSV infection. Based on these data, we speculate the virus-induced increase in $[Ca^{2+}]_i$ mediated by TRPV1 activation may contribute to the clinical manifestations of asthma, including the increased synthesis and release of Th2 cytokines, mucus overproduction, breakdown in barrier permeability, and increased bronchoconstriction (see **Table 1** for summary of RSV effects on **β_2AR**).

In addition to the asthma status, the patient's age also affected TRVP1 expression during RSV infection (17). In fact, by comparing the TRPV1-mediated Ca^{2+} influx in human bronchial epithelial cells from children and adults, we noted that expression, localization, and activity of this channel were increased during RSV infection of cells harvested from children but not adults, suggesting that RSV entry and replication is more efficient in the bronchial epithelium early in life. The data summarized in the previous paragraphs are timely and important because of the increased morbidity for RSV bronchiolitis and asthma among pediatric patients (18). In addition, this study can shed light into the limited efficacy of currently available therapies (19). More importantly, pharmacological inhibition of the NGF-TrkA-$TRPV_1$ axis shows promise as a novel strategy to limit the impact of RSV infections in both asthmatic and non-asthmatic children.

Smooth muscle

Recent evidence shows that RSV can infect and alter the structure and function of human airway smooth muscle (ASM) cells, in particular by disrupting the structure, distribution, and function of β_2 adrenergic

Table 1. *Effects of RSV on $TRPV_1$ channels.*

	RSV EFFECTS ON TRPV1 CHANNELS
1	The lower airway epithelium and smooth muscle from children with asthma displays elevated basal and RSV-induced $TRPV_1$ expression compared with nonasthmatic controls.
2	RSV increases $TRPV_1$ expression and activation as a consequence of increased NGF-TrkA signaling.
3	TRPV1- mediated increase in $[Ca^{2+}]_i$ can originate from either the extra-cellular pool or intracellular stores depending on asthma status and RSV infection.
4	RSV-induced increase in $[Ca^{2+}]_i$ mediated by $TRPV_1$ activation contributes to the clinical manifestations of asthma, including: – increased synthesis and release of TH_2 cytokines; – mucus overproduction; – breakdown in barrier permeabilit; – increased bronchoconstriction.

Adapted from: Harford TJ et al. Asthma predisposition and respiratory syncytial virus infection modulate transient receptor potential vanilloid 1 function in children's airways. J Allergy Clin Immunol. 2018;141(1):414-6.e4 and Harford TJ et al. RSV infection potentiates TRPV1-mediated calcium transport in bronchial epithelium of asthmatic children. Am J Physiol Lung Cell Mol Physiol. 2021;320(6):L1074-L1084.

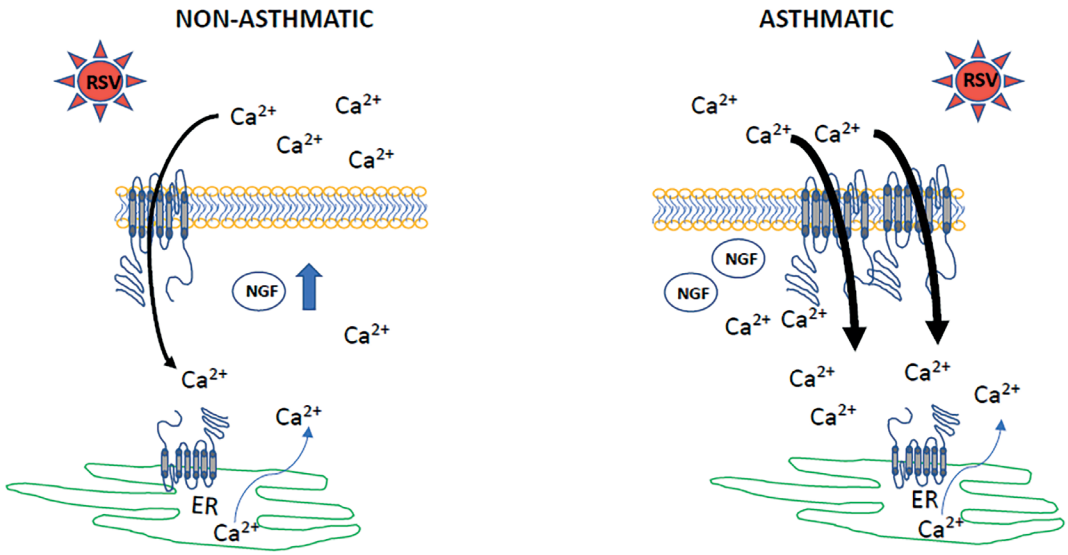

Figure 1. *Modulation of $TRPV_1$-mediated Ca^{2+} influx in Human Bronchial Epithelial (HBE) cells. RSV infection of non-asthmatic airway epithelial cells in HBE cells activates NGF protein expression. Increased NGF expression results in a signaling cascade that leads to increased $TRPV_1$ expression and $TRPV_1$ translocation to the plasma membrane, where it can be stimulated by multiple physical or chemical irritants and initiate inward transfer of Ca^{2+} from the extracellular compartment to the cytosol. In contrast, basal expression of NGF is already increased at rest in airway epithelial cells from asthmatic children, which results in more $TRPV_1$ channels at the plasma membranes and lower threshold of activation. Additionally, there is increased expression of intracellular $TRPV_1$ localized to the ER, where it can release Ca^{2+} from the intracellular storage sites into the cytosol and lead to higher cytosolic Ca^{2+} at rest. During RSV infection there is a shift of Ca^{2+} influx from both intracellular stores and extracellular sources to primarily from extracellular sources. Increased intracellular calcium leads to increased mucus production, bronchoconstriction, airway barrier permeability, and increased cytokine production resulting in the asthma phenotype.*
(Adapted from: Harford et al., Am J Physiol Lung Cell Mol Physiol. 2021;320(6):L1074-L1084).

receptors (β_2AR) (20). β_2AR are transmembrane glycoprotein receptors coupled with guanine nucleotide (GTP) binding proteins (G proteins) and classified into three subtypes β_1, β_2, β_3 [51]. β_2AR is composed of 8 alpha helices (3 extracellular and 5 intracellular), is bound to cellular membranes, and signals intracellularly through heterotrimeric GS proteins consisting of alpha, beta, and gamma subunits (21) (**Figure 2**). This receptor is encoded on chromosome 5 and expressed on various cell types, including epithelial and endothelial cells, eosinophils, lymphocytes, mast cells, mucous glands, and especially skeletal and smooth muscle.

Because of the sparse sympathetic innervation of human airways, endogenous catecholamines secreted from the adrenal medulla (*e.g.*, epinephrine and norepinephrine) are primarily responsible for β_2AR stimulation. However, the same pathway has been extensively targeted for therapeutic purposes using synthetic compounds able to bind the receptor with higher selectivity, and usually classified according to the duration of their bronchodilator activity into short-acting and long-acting (22). Following binding of agonist ligands to the β_2AR, the alpha subunit of coupled GS proteins promotes the conversion of adenosine triphosphate (ATP) into cAMP, which through the catalytic subunit of protein kinase-A (PKA) reduces the intracellular Ca^{2+} concentration, promotes smooth muscle relaxation, and prevents muscle contraction. Hence, short acting β2AR agonists (*e.g.*, albuterol) have been for decades the most effective and widely used medicines to relieve acute airway obstruction during an asthma exacerbation, whereas long- acting agonists (*e.g.*, salmeterol, formoterol) have been widely used as controller agents to prevent bronchospasm in chronic asthma patients.

The clinical manifestations of viral bronchiolitis in infants and young children are virtually identical to those of an asthma attack (*i.e.*, wheezing, cough, and increased work of breathing), and fully developed ASM (23) expressing of fully functional β_2AR receptors has been demonstrated from the first years of life (24). Yet,

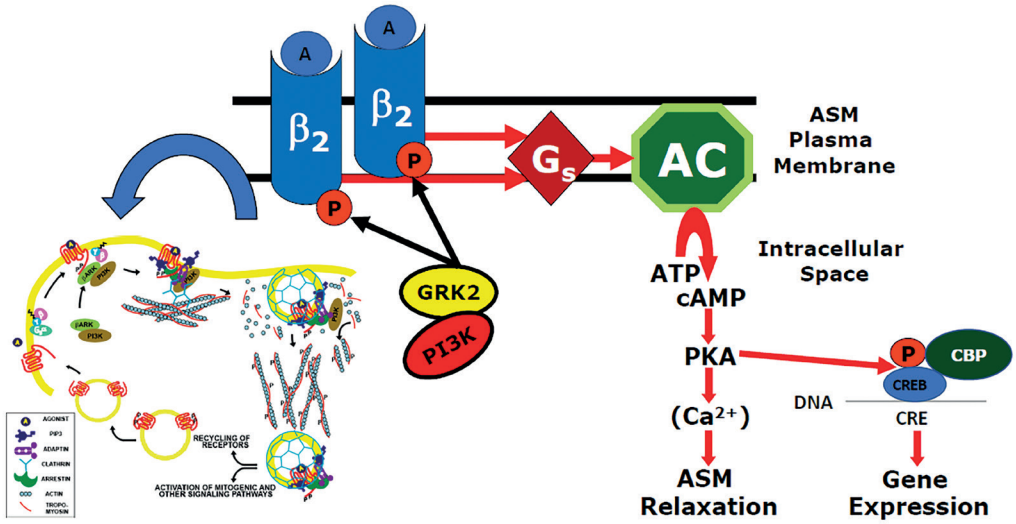

Figure 2. *The Beta2-adrenergic Receptor (β2AR) Pathway. β2AR is a prototypical member of the heptahelical transmembrane family of G protein-coupled receptors (GPCRs). This family includes three receptor subtypes, β1, β2, and β3, with β2AR having predominant expression in the respiratory tract. Activation of βARs by binding to b-agonists results in the activation of a heterotrimeric stimulatory G protein (GS) and release of its alpha and beta-gamma subunits. The alpha subunit then activates the enzyme adenylyl cyclase (AC), which generates the second messenger cyclic adenosine monophosphate (cAMP), which, in turn, mediates the activation of protein kinase A (PKA). Activated β2ARs are phosphorylated and desensitized by GPCR kinases (GRKs) and PKA. When activated by cAMP, PKA stimulates downstream signaling events that mediate the relaxation of airway smooth muscle cells in the respiratory tract by inhibition of calcium-activated contraction. PKA also phosphorylates the cAMP response element (CRE) binding protein (CREB), which results in its binding to CREB binding protein (CBP). CBP binding leads to recruitment of the basal transcriptional apparatus resulting in gene transcription.*

multiple clinical trials have failed to demonstrate any clinical benefit of β2AR agonists in infants with RSV bronchiolitis (25), and systematic metanalysis has concluded that β_2AR agonists are clearly less effective in the setting of viral respiratory infections, especially when airway obstruction is caused by RSV infection (26). Furthermore, a recent report indicates that virus-infected asthmatic children have higher risk of treatment failure during acute exacerbations (27).

Moore *et al.* investigated the effect of RSV on β_2AR responsiveness by measuring isoproterenol (ISO)-mediated cAMP synthesis, β_2AR density, and GI protein expression in human ASM cells incubated with RSV (20). This study showed that RSV infection of human ASM cells inhibits ISO-induced cAMP synthesis in a time- and dose-dependent manner and reduces β_2AR density on cell membranes. Consistently, RSV reduced airway responses to β_2AR-agonists, both directly and indirectly by inducing heterologous keratinocyte cytokine (KC)/CXCR2-mediated desensitization (28). Interestingly, in this study β_2AR desensitization occurred in the absence of receptor internalization or degradation; rather, it resulted from receptor uncoupling due to phosphorylation by GRK-2.

More recently, Harford *et al.* investigated the expression, structure, and activity of β_2AR in primary human ASM cells derived from children's lung tissues and infected *ex vivo* with RSV (29). This study showed conclusively that RSV infection triggers proteolytic cleavage of β_2AR, which is mediated by the intracellular proteasome and results in net loss of functional receptors on ASM membranes, consequently reducing ASM relaxation in response to exogenous β_2AR agonists like albuterol (**Figure 3**). Simultaneously, RSV activates multiple pathways favoring airway obstruction, such as non-canonical activation of adenylyl cyclase resulting in less robust cAMP synthesis and increased cytosolic calcium concentration initiating smooth muscle contraction (see **Table 2** for summary of RSV effects on β_2AR). Collectively, these findings not only provide a suitable mechanism for the reported lack of clinical efficacy of β_2AR agonists in the setting of virus-induced wheezing, but also open the path to developing more precise therapeutic strategies in the future.

Peripheral nerves

Several prospective epidemiologic studies have linked RSV infection in early life to persistent airway hyper-

reactivity and inflammation, which is likely to involve a complex dysregulation of peripheral neural control (4). Airway patency depends on the activity and interaction between adrenergic, cholinergic, and nonadrenergic–noncholinergic (NANC) pathways primarily bundled within the vagus nerve. Albeit adrenergic fibers are remarkably sparse in the human smooth muscle, these nerves release catecholamines and promote bronchorelaxation. Cholinergic fibers release acetylcholine and are a primary effector of bronchoconstriction in response to chemical, physical, and pharmacologic stimulation. NANC pathways comprise inhibitory (NANCi) and excitatory (NANCe) sub-systems. The former promotes ASM relaxation, which is mediated by the release of vasoactive intestinal peptide (VIP) and nitric oxide (NO). The latter is primarily constituted by unmyelinated (C-type) sensory nerve fibers and causes bronchoconstriction via release of tachykinins like substance P, neurokinin A, and neurokinin B.

Neurokinins selectively bind 3 receptors with a rhodopsin-like structure, NK-1, NK-2, and NK-3, also expressed on epithelial and immune cells. More specifically, NK-1 has high affinity for substance P and mediates its pro-inflammatory and immunomodulatory effects, including increased endothelial permeability; proliferation and activation of T cells, B lymphocytes, monocytes, and macrophages; and mast cells degranulation. The same cell types expressing NK-1 receptors also support the enzymatic activities of neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE), two surface-bound peptidases that cleave the carboxyl-terminal dipeptide of substance P, thereby inhibiting its actions. Among many other studies exploring neurogenic-mediated inflammation, my lab showed that corticosteroids prevent airway edema by upregulating peptidase activity, a potent anti-inflammatory effect that was completely reversed only when both NEP and ACE were simultaneously inhibited (30, 31).

Upstream from the NANCe system, we also reported that RSV upregulates the expression and activation of nerve growth factor (NGF) and its TrkA and $p75^{NTR}$ receptors both *in vitro*, *in vivo* animal models, and, most importantly, in the lower airways of human infants with RSV- positive bronchiolitis (32). Together

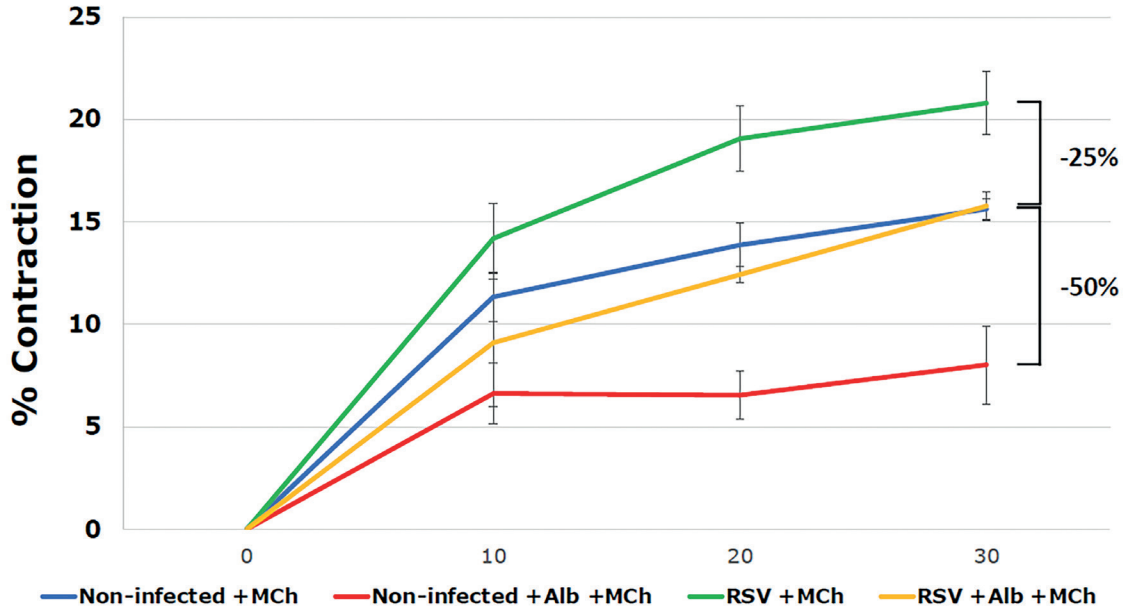

Figure 3. *Loss of Response to β2AR Agonist in RSV infection. Human airway smooth muscle cells (HASMCs) embedded in a 1.5% collagen matrix were incubated with sterile medium or RSV for 48 hours before being subjected to a 30-min treatment with vehicle or 200 μM albuterol followed by 200 μM methacholine. Methacholine induced a 20% contraction in RSV-infected cells, as compared to 15% contraction in uninfected cells. Pretreatment with albuterol led to a 50% reduction in the methacholine-induced contraction of uninfected cells (8% contraction), whereas methacholine-induced contraction was reduced by 25% (15% contraction) in RSV-infected cells pretreated with albuterol.*
(Adapted from: Harford TJ et al. Respiratory syncytial virus induces β2-adrenergic receptor dysfunction in human airway smooth muscle cells. Sci Signal. 2021;14(685):eabc1983).

with brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT-4/5), NGF is a prototypical neurotrophin critically involved in the modulation of neuronal development, survival, and function, including synapse formation and plasticity (33). NGF-mediated biological activities result from differential activation of its putative high-affinity receptor TrkA of the tropomyosin-related kinase (Trk) family, as well as the p75 pan-neurotrophin receptor (p75NTR), a member of the TNF receptor superfamily that binds with low-affinity the mature form of NGF and much higher affinity its precursor proNGF. Depending on the relative abundance and distribution of receptors and associated co-factors, NGF can mediate neurite outgrowth, migration, survival, cell cycle arrest, and apoptosis. The other Trk receptors, TrkB and TrkC, bind with high affinity BDNF and NT3/4 respectively and lead to activation of several downstream signaling cascades, including the PI3K/Akt (protein kinase B, PKB) and phospholipase Cγ (PLC) pathways, which in turn promote neuronal development, axon and dendrite outgrowth, membrane trafficking, glial differentiation, and interactions with adjacent neurons.

The crucial role of neurotrophic proteins and their specific receptors in the pathophysiology of RSV-mediated airway inflammation and hyperreactivity has been demonstrated by my lab and others in several publications. For instance, increased NGF protein levels and TrkA expression were detected in macrophages and airway epithelial cells in the BAL of infants with acute RSV infection requiring ventilatory support (32). In addition, after RSV infection NGF promoted overgrowth of neurites with higher substance P content, heightened nociceptive sensory fibers' responsiveness, activated release of acetylcholine and pro-inflammatory peptides, and induced long-term remodeling of NANC in the airway (4). Finally, NGF over-expression might further affect ASM tone dysregulation via a decrease in catecholamine production, resulting from differentiation of adrenal medulla cells into neuronal cells (34).

Slow-conducting non-myelinated C-fibers represent up to 75% of vagal bronchopulmonary afferents. They innervate the airways from the upper (nose, larynx, trachea) to the lower tract, including the parenchyma and alveolar wall. Critical to their function is the expression of TRP ion channels (discussed also in the section dedicated to the epithelium), consisting of 28 members classified based on their structure and activation mechanism into six subgroups: ankyrin (TRPA), canonical (TRPC), melastatin (TRPM), mucolipin (PRTML), polycystin (TRPP), and vanilloid (TRPV) (35). In particular, the six members of the TRPV subfamily (TRPV1–6) are nonselective cationic ligand-gated channels with high permeability to Ca^{2+}. In addition to nociceptive C-fibers, these channels are commonly expressed by a variety of non-neuronal cells, including airway epithelial and immune cells (36). TRPV channels activation is triggered by physical and chemical stimuli, both exogenous (temperature, osmolarity, airborne pollutants, cigarette smoke, allergens, alkaloids) and endogenous (thromboxanes, prostaglandins, leukotrienes, and other arachidonic acid derivatives). Following exogenous or endogenous stimulation, TRPVs allow extracellular Ca^{2+} entrance into neuronal and non-neuronal target cells, which, in turn, leads to the

Table 2. *Effects of RSV on β_2AR receptors.*

	RSV EFFECTS ON β2AR RECEPTORS
1	RSV infection of human airway smooth muscle cells in children results in: – phosphorylation of β_2Ars; – loss of β_2ARs from the plasma membrane; – proteasomal cleavage of β_2Ars; – increased Ca^{2+} signaling promoting contractility.
2	RSV infection also leads to non-canonical AC activation leading to cAMP production and CREB phosphorylation independent of β_2AR stimulation and without causing ASM relaxation.
3	The β_2AR pathway may be required for sustenance of RSV infection, as selective blockers inhibit viral replication.
4	Dysregulation of the location, abundance, and function of the β_2AR by RSV underlie the ineffectiveness of β_2AR agonists in relieving airway obstruction in infected patients.

Harford TJ et al. Respiratory syncytial virus induces β_2-adrenergic receptor dysfunction in human airway smooth muscle cells. Sci Signal. 2021;14(685):eabc1983.

activation of a number of proinflammatory and defense mechanisms (37). Moreover, TRPVs act as receptors for "damage signals" able to transfer the signal from neuronal fibers to the immune cells, thus inducing and perpetuating a pro-inflammatory status (38).

FUTURE PROSPECTIVE

RSV infection remains the leading cause of lower respiratory tract infections in infants and young children. Despite strong epidemiologic evidence showing a close relationship between RSV infection and the subsequent development of recurrent wheezing and asthma, the exact molecular mechanisms underlying the chronic sequelae of the infection remain to be fully elucidated. There is no doubt that RSV drives complex modifications of the host's local and systemic immune response. In particular, it has been proposed that post-RSV asthma is associated with an atopic phenotype because Th2-biased immune responses have been reported. However, RSV also interacts with a number of non-immune systems and mechanisms, such as the airways' neural networks. Specifically, RSV makes the airways abnormally susceptible to neurogenic inflammation by upregulating the NGF-TrkA axis, which in turn increases the expression of substance P and its cognate NK1 receptor density on target cells, including lymphocytes, macrophages, mast cells, and endothelial cells. NGF also modulates the expression of $TRPV_1$ channels on sensory nerves, airway epithelial and smooth muscle cells, thereby heightening the inflammatory and bronchospastic response of the airways to chemical (*e.g.*, lower pH caused by gastroesophageal reflux), physical (*e.g.,* cooling of the airway mucosa caused by hyperventilation during exercise), and other inflammatory stimuli.

In summary, RSV can establish crucial interactions between the airway's neural network and the immune system that result in long-term airway dysfunction and predispose to the onset and maintenance of persistent airway inflammation and hyperreactivity. These interactions are influenced not only by the viral pathogens and other environmental agents but also by a constellation of host factors, such as genetic susceptibility that can affect the efficiency of antiviral defenses, viral replication, and virus-mediated injury to the airways. All these variables justify the individual variability in the severity of infection, damage extension, duration, and magnitude of RSV effects in the pediatric population. Due to the complexity of pathophysiologic mechanisms involved in the RSV infection, it is reasonable for future management strategies to be focused on modulating the interactions between the virus, host immune response, and neuronal pathways.

Currently, the treatment of RSV disease is limited to supportive care, and the only prevention strategy is passive prophylaxis with a humanized monoclonal antibody (palivizumab) indicated only in early preterm newborns and those with underlying cardio-pulmonary conditions. A vaccine for active immunization is not currently available because its development has been stifled by several challenges, particularly the immaturity of the host immune response during the first 3 months of life, when the infection peaks. The two most promising strategies being pursued for the immediate future are maternal immunization and new long-active monoclonal antibodies (mAbs). Maternal immunization offers the advantage of being completely safe for the offspring and allow the passage of protective IgG into the fetus, able to protect already at birth and for the very first months of life when the risk of severe infections is especially high. The main disadvantage is that maternal IgG cannot cross the placenta efficiently before the last trimester of gestation, and therefore infants born prematurely, who are at very high risk for severe infection, will not be protected by maternal vaccination.

Long-acting monoclonal antibodies are currently being reviewed by the U.S. Federal Drug Administration (FDA) and seems to provide consistent protection against RSV for at least five months, thus covering the entire duration of the RSV season and offering greater flexibility in the timing of administration. It is reasonable to predict that this new generation of monoclonals will be available to all infants regardless of gestational age, presence of comorbidities, and maturity of the immune system. If so, we should see a dramatic decline of the frequency, morbidity, and mortality related to this infection, which is still the most common cause of hospitalization for infants in the developed countries, as well as an important cause of infant mortality in developing countries. In addition, because of the well-established epidemiologic link between early-life RSV bronchiolitis, airway modeling, and asthma pathogenesis in later childhood, we would expect to witness significant reduction in the prevalence of childhood asthma, which is currently the most common chronic pediatric disease worldwide.

ACKNOWLEDGEMENTS

The research described in this article has been funded over the years by grants of the National Heart, Lung, and Blood Institute to Prof. Giovanni Piedimonte.

COMPLIANCE WITH ETHICAL STANDARDS

Conflicts of interests

The Author has declared no conflict of interests.

Financial support

The study has been supported by the United States National Heart, Lung & Blood Institute.

Authorship

Professor Giovanni Piedimonte is the sole Author of this manuscript.

Author contributions

N/A.

Ethical approval

Human studies and subjects

Human studies were performed in accordance with local IRB-approved protocols.

Animal studies

Animal studies were performed in accordance with local IACUC-approved protocols.

Data sharing and data accessibility

N/A.

Publication ethics

Plagiarism

All original studies are cited as appropriate.

Data falsification and fabrication

All the data correspond to the real.

REFERENCES

1. Piedimonte G, Perez MK. Respiratory syncytial virus infection and bronchiolitis. Pediatr Rev. 2014;35(12):519-30. doi: 10.1542/pir.35-12-519. Erratum in: Pediatr Rev. 2015;36(2):85.
2. Piedimonte G. Respiratory syncytial virus and asthma: speed-dating or long-term relationship? Curr Opin Pediatr. 2013;25:344-9. doi: 10.1097/MOP.0b013e328360bd2e.
3. Openshaw P, Murphy EE, Hosken NA, Maino V, Davis K, Murphy K, et al. Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 populations. J Exp Med. 1995;182:1357-67. doi: 10.1084/jem.182.5.1357.
4. Piedimonte G. Neural mechanisms of respiratory syncytial virus-induced inflammation and prevention of respiratory syncytial virus sequelae. Am J Respir Crit Care Med. 2001;163: S18-S21. doi: 10.1164/ajrccm.163.supplement_1.2011113.
5. Colten HR, Krause JE. Pulmonary inflammation--a balancing act. N Eng J Med. 1997;336:1094-6. doi: 10.1056/NEJM199704103361511.
6. Liesman RM, Buchholz UJ, Luongo CL, Yang L, Proia AD, DeVincenzo JP, et al. RSV-encoded NS2 promotes epithelial cell shedding and distal airway obstruction. J Clin Inv. 2014;124:2219-33. doi: 10.1172/JCI72948.
7. Weltzin R, Hsu SA, Mittler ES, Georgakopoulos K, Monath TP. Intranasal monoclonal immunoglobulin A against respiratory syncytial virus protects against upper and lower respiratory tract infections in mice. Antimicrob Agents Chemother. 1994; 38: 2785-91. doi: 10.1128/AAC.38.12.2785.
8. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004; 303:1532-5. doi: 10.1126/science.1092385.
9. Emboriadou M, Hatzistilianou M, Magnisali CH, Sakelaropoulou A, Exintari M, Conti P, et al. Human neutrophil elastase in RSV bronchiolitis. Ann Clin Lab Sci. 2007;37:79-84. Available from: https://pubmed.ncbi.nlm.nih.gov/17311874/. Accessed: Oct 11, 2022.
10. Harrison AM, Bonville CA, Rosenberg HF, Domachowske JB. Respiratory syncytical virus- induced chemokine expression in the lower airways: eosinophil recruitment and degranulation. Am J Respir Crit Care Med. 1999;159:1918-24. doi: 10.1164/ajrccm.159.6.9805083.
11. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature. 1997;389(6653):816-24. doi: 10.1038/39807.
12. Watanabe N, Horie S, Michael GJ, Keir S, Spina D, Page CP, et al. Immunohistochemical co-localization of transient receptor potential vanilloid (TRPV)1 and sensory neuropeptides in the guinea-pig respiratory system. Neuroscience. 2006;141:1533-43. doi: 10.1016/j.neuroscience.2006.04.073.
13. Jia Y, Lee LY. Role of TRPV receptors in respiratory diseases. Biochim Biophys Acta. 2007; 1772: 915-27. doi: 10.1016/j.bbadis.2007.01.013.
14. Piedimonte G, Rodriguez MM, King KA, McLean S, Jiang X. Respiratory syncytial virus upregulates expression of the substance P receptor in rat lungs. Am J Physiol Lung Cell Mol Physiol. 1999;277:L831–L840. doi: 10.1152/ajplung.1999.277.4.L831.

15. Omar S, Clarke R, Abdullah H, Brady C, Corry J, Winter H, et al. Respiratory virus infection up-regulates TRPV1, TRPA1 and ASICS3 receptors on airway cells. PLOS One. 2017;12: e0171681. doi: 10.1371/journal.pone.0171681.
16. Harford TJ, Grove L, Rezaee F, Scheraga R, Olman MA, Piedimonte G. RSV infection potentiates TRPV1-mediated calcium transport in bronchial epithelium of asthmatic children. Am J Physiol Lung Cell Mol Physiol. 2021;320:L1074-L1084. doi: 10.1152/ajplung.00531.2020.
17. Harford TJ, Rezaee, F, Scheraga RG, Olman MA, Piedimonte, G. Asthma predisposition and respiratory syncytial virus infection modulate transient receptor potential vanilloid 1 function in children's airways. J Allergy Clin immunol. 2018;141(1): 414-6.e4. doi: 10.1016/j.jaci.2017.07.015.
18. Hall CB, Weinberg GA, Blumkin AK, Edwards KM, Staat MA, Schultz AF, et al. Respiratory syncytial virus-associated hospitalizations among children less than 24 months of age. Pediatrics. 2013; 132(2):e341-8. doi: 10.1542/peds.2013-0303.
19. Merckx J, Ducharme FM, Martineau C, Zemek R, Gravel J, Chalut D, et al. Respiratory viruses and treatment failure in children with asthma exacerbation. Pediatrics. 2018;142(1):e20174105. doi: 10.1542/peds.2017-4105.
20. Moore PE, Cunningham G, Calder MM, DeMatteo AD Jr, Peeples ME, Summar ML, et al. Respiratory syncytial virus infection reduces beta2-adrenergic responses in human airway smooth muscle. Am J Respir Cell Mol Biol. 2006 Nov;35(5):559-64. doi: 10.1165/rcmb.2005-0282OC.
21. Johnson M. The beta-adrenoceptor. Am J Respir Crit Care Med. 1998;158(5 Pt 3):S146-53. doi: 10.1164/ajrccm.158.supplement_2.13tac110.
22. Johnson M. Molecular mechanisms of beta(2)-adrenergic receptor function, response, and regulation. J Allergy Clin Immunol. 2006;117(1):18-24; quiz 25. doi: 10.1016/j.jaci.2005.11.012. PMID: 16387578.
23. Roesler AM, Wicher SA, Ravix J, Britt RD Jr, Manlove L, Teske JJ, et al. Calcium sensing receptor in developing human airway smooth muscle. J Cell Physiol. 2019;234:14187-97. 2019;234(8):14187-97. doi: 10.1002/jcp.28115.
24. Goldstein AB, Castile RG, Davis SD, Filbrun DA, Flucke RL, McCoy KS, et al. Bronchodilator responsiveness in normal infants and young children. Am J Respir Crit Care Med. 2001;164(3):447-54. doi: 10.1164/ajrccm.164.3.2005080.
25. Ralston SL, Lieberthal AS, Meissner HC, Alverson BK, Baley JE, Gadomski AM, et al. American Academy of Pediatrics clinical practice guideline: the diagnosis, management, and prevention of bronchiolitis. Pediatrics. 2014;134(5):e1474-502. doi: 10.1542/peds.2014-2742. Erratum in: Pediatrics. 2015;136(4):782.
26. Gadomski AM, Scribani MB. Bronchodilators for bronchiolitis. Cochrane Db Syst Rev 2014; CD001266. 2014;2014(6):CD001266. doi: 10.1002/14651858.CD001266.pub4.
27. Merckx J, Ducharme FM, Martineau C, Zemek R, Gravel J, Chalut D, et al. Respiratory viruses and treatment failure in children with asthma exacerbation. Pediatrics. 2018;142(1):e20174105. doi: 10.1542/peds.2017-4105.
28. Davis IC, Xu A, Gao Z, Hickman-Davis JM, Factor P, Sullender WM, et al. Respiratory syncytial virus induces insensitivity to beta-adrenergic agonists in mouse lung epithelium in vivo. Am J Physiol Lung Cell Mol Physiol. 2007;293(2): L281–L289. doi: 10.1152/ajplung.00458.2006.
29. Harford TJ, Rezaee F, Gupta MK, Bokun V, Naga Prasad SV, Piedimonte G. Respiratory syncytial virus induces β2-adrenergic receptor dysfunction in human airway smooth muscle cells. Sci Signal 2021; 14: eabc1983. Sci Signal. 2021;14(685):eabc1983. doi: 10.1126/scisignal.abc1983.
30. Piedimonte G, McDonald DM, Nadel JA. Glucocorticoids inhibit neurogenic plasma extravasation and prevent virus-potentiated extravasation in the rat trachea. J Clin Investig. 1990;86(5):1409-15. doi: 10.1172/JCI114855.
31. Piedimonte G, McDonald DM, Nadel JA. Neutral endopeptidase and kininase II mediate glucocorticoid inhibition of neurogenic inflammation in the rat trachea. J Clin Investig. 1991;88(1):40-4. doi: 10.1172/JCI115302.
32. Tortorolo L, Langer A, Polidori G, Vento G, Stampachiacchere B, Aloe L, et al. Neurotrophin overexpression in lower airways of infants with respiratory syncytial virus infection. Am J Respir Crit Care Med. 2005;172(2):233-7. doi: 10.1164/rccm.200412-1693OC.
33. Manti S, Brown P, Perez MK, Piedimonte G. The role of neurotrophins in inflammation and allergy. Vitam Horm. 2017;104:313-41. doi: 10.1016/bs.vh.2016.10.010.
34. Rezaee F, Gibson LF, Piktel D, Othumpangat S, Piedimonte G. Respiratory syncytial virus infection in human bone marrow stromal cells. Am J Respir Cell Mol Biol. 2011;45(2):277-86. doi: 10.1165/rcmb.2010-0121OC.
35. Pedersen SF, Owsianik G, Nilius B. TRP channels: an overview. Cell Calcium. 2005;38(3-4):233-52. doi: 10.1016/j.ceca.2005.06.028.
36. Groneberg DA, Niimi A, T. DQ, B. C, Hew. M, Fischer A, Chung KF. Increased expression of transient receptor potential vanilloid-1 in airway nerves of chronic cough. Am J Respir Crit Care Med 2004; 170: 1276-80.
37. Du Q, Liao Q, Chen C, Yang X, Xie R, Xu J. The role of transient receptor potential vanilloid 1 in common diseases of the digestive tract and the cardiovascular and respiratory system. Front Physiol. 2019;10: 1064. 2019;10:1064. doi: 10.3389/fphys.2019.01064.
38. Tränkner D, Hahne N, Sugino K, Hoon MA, Zuker C. Population of sensory neurons essential for asthmatic hyperreactivity of inflamed airways. Proc Natl Acad Sci. 2014;111(31):11515-20. doi: 10.1073/pnas.1411032111.