



## OPEN ACCESS

EDITED BY  
Katarina Polcicova,  
Slovak Academy of Sciences, Slovakia

\*CORRESPONDENCE  
Juan C. Hernandez,  
juankhernandez@gmail.com

RECEIVED 08 December 2022  
ACCEPTED 28 June 2023  
PUBLISHED 24 August 2023

CITATION  
Correa D, Giraldo DM, Gallego S,  
Taborda NA and Hernandez JC (2023),  
Immunity towards human respiratory  
syncytial virus.  
*Acta Virol.* 67:11887.  
doi: 10.3389/av.2023.11887

COPYRIGHT  
© 2023 Correa, Giraldo, Gallego,  
Taborda and Hernandez. This is an  
open-access article distributed under  
the terms of the [Creative Commons  
Attribution License \(CC BY\)](#). The use,  
distribution or reproduction in other  
forums is permitted, provided the  
original author(s) and the copyright  
owner(s) are credited and that the  
original publication in this journal is  
cited, in accordance with accepted  
academic practice. No use, distribution  
or reproduction is permitted which does  
not comply with these terms.

# Immunity towards human respiratory syncytial virus

Dahiana Correa<sup>1</sup>, Diana M. Giraldo<sup>1</sup>, Salomon Gallego<sup>2</sup>,  
Natalia A. Taborda<sup>2,3</sup> and Juan C. Hernandez<sup>1,4\*</sup>

<sup>1</sup>Infettare, Facultad de Medicina, Universidad Cooperativa de Colombia, Medellín, Colombia, <sup>2</sup>Grupo de Investigaciones Biomédicas Uniremington, Programa de Medicina, Facultad de Ciencias de la Salud, Corporación Universitaria Remington, Medellín, Colombia, <sup>3</sup>Universidad Cooperativa de Colombia, Campus Medellín, Envigado, Colombia, <sup>4</sup>Grupo Inmunovirología, Facultad de Medicina, Universidad de Antioquia UdeA, Medellín, Colombia

Respiratory infections remain a significant cause of morbidity and mortality, becoming a serious public health issue worldwide. The human respiratory syncytial virus (hRSV) is still one of the most relevant pathogenic agents involved in respiratory infections in children, the leading cause of bronchiolitis worldwide. In most cases, hRSV infection is not complicated; however, limited treatment and vaccine options increase the morbidity rates associated with bronchiolitis. The innate immune response governs the severity of the disease and controls the viral infection outcome. Current knowledge about the mechanisms involved in viral PAMPs (pathogen-associated molecular pattern molecules) recognition, genetic characteristics of the inflammatory response, and understanding of antiviral response is crucial for vaccine development and biomarker tools to predict complications and guide therapeutic management. Here, we review key concepts related to pathogenesis and immune response against hRSV, highlighting aspects that could be considered in vaccine development.

## KEYWORDS

human respiratory syncytial virus (hRSV), bronchiolitis, immune response, inflammation, pathogenesis

## Introduction

Human respiratory syncytial virus (hRSV) is the primary cause of severe lower respiratory tract infection among newborns and young children worldwide (Shi et al., 2017). hRSV infection also includes upper respiratory tract infections, aggravation of asthma, and wheeze induced by the hRSV (Barr et al., 2019). The hRSV risk of infection is over 60%–70% in the first year of life and nearly 100% by 2 or 3 years of age (Meng et al., 2014).

In 2015, there have been an estimated 33.1 million hRSV-related lower respiratory tract infections, 3.2 million hRSV-related hospitalizations, and 59,600 deaths in children under 5 years of age, with an overall mortality of 118,200 (Shi et al., 2017). This virus is ubiquitously transmitted, being a risk factor for people with immunodeficiencies and elderly individuals. Further, hRSV is an essential nosocomial infection agent (Falsey et al., 2005; Gelfand, 2012; Checchia et al., 2017).

hRSV is an enveloped, single-stranded, and negative-sense RNA virus classified into the family *Pneumoviridae*, genus *Orthopneumovirus*, species human *orthopneumovirus* (Hall, 2001; Afonso et al., 2016). The viral genome contains ten genes encoding for 11 proteins: fusion protein (F); the glycoprotein (G); the small hydrophobic protein (SH); nucleoprotein (N); phosphoprotein (P); large protein (L); matrix (M), M2-1 and M2-2 regulatory proteins; non-structural (NS) proteins (NS1 and NS2). All these proteins are critical for viral replication and are involved in the innate immunity response (Cao et al., 2021).

Two hRSV subtypes (A and B) have been identified, which are phylogenetically and antigenically different. Most of the variability has been detected in the gene encoding the G protein, which is the most variable protein of the virus (Peret et al., 1998; Griffiths et al., 2017; Hu et al., 2017). Variations in hRSV-A and hRSV-B are associated with evolutionary mechanisms characterized by the induction of anti-G antibodies against primary epitopes (Botosso et al., 2009). hRSV-A and hRSV-B groups have cocirculated during specific seasonal epidemics but can circulate independently in human populations (Pangesti et al., 2018). Some studies report a higher prevalence and severe clinical course in hRSV-A (Jafri et al., 2013; Tabarani et al., 2013; Shen et al., 2022), but the evidence is contradictory (McIntosh et al., 1993; Devincenzo, 2004; Laham et al., 2017).

## Pathogenesis

Inoculation of aerosol particles or direct contact facilitates virus entry via the nasopharynx, spreading to the lower respiratory tract toward the bronchioles (Piedimonte and Perez, 2014; Battles and McLellan, 2019). During the viral replication, the G-protein is responsible for the attachment to ciliated epithelial cells through the CX3CR1 receptor (Levine et al., 1987; Johnson et al., 2015; Zhivaki et al., 2017); the pre-F-protein allows the fusion of the viral envelope with the host cell membrane and hRSV enters by endocytosis (Tayyari et al., 2011). As soon as the virus is in the cytoplasmic inclusion bodies, it replicates its genome using the viral RNA-dependent RNA polymerase (RdRp) complex (large protein-L and the phosphoprotein-P) (Sourimant et al., 2015). The viral protein M2-1 is added to the complex to act as a cofactor of the transcriptional process (Sourimant et al., 2015; Braun et al., 2021).

Different receptors have been involved in the early immune response via NF- $\kappa$ B and IFN response factors (Liu et al., 2007; Okabayashi et al., 2011; Zeng et al., 2012), including epidermal growth factor (EGF) receptor (Weigl et al., 2001), intercellular adhesion molecule 1 (ICAM-1) (Law et al., 2004), annexin II (Fjaerli et al., 2004), calcium-dependent lectins (Fjaerli et al., 2004), and heparan sulfate proteoglycans (HSPGs) (Bradley et al.,

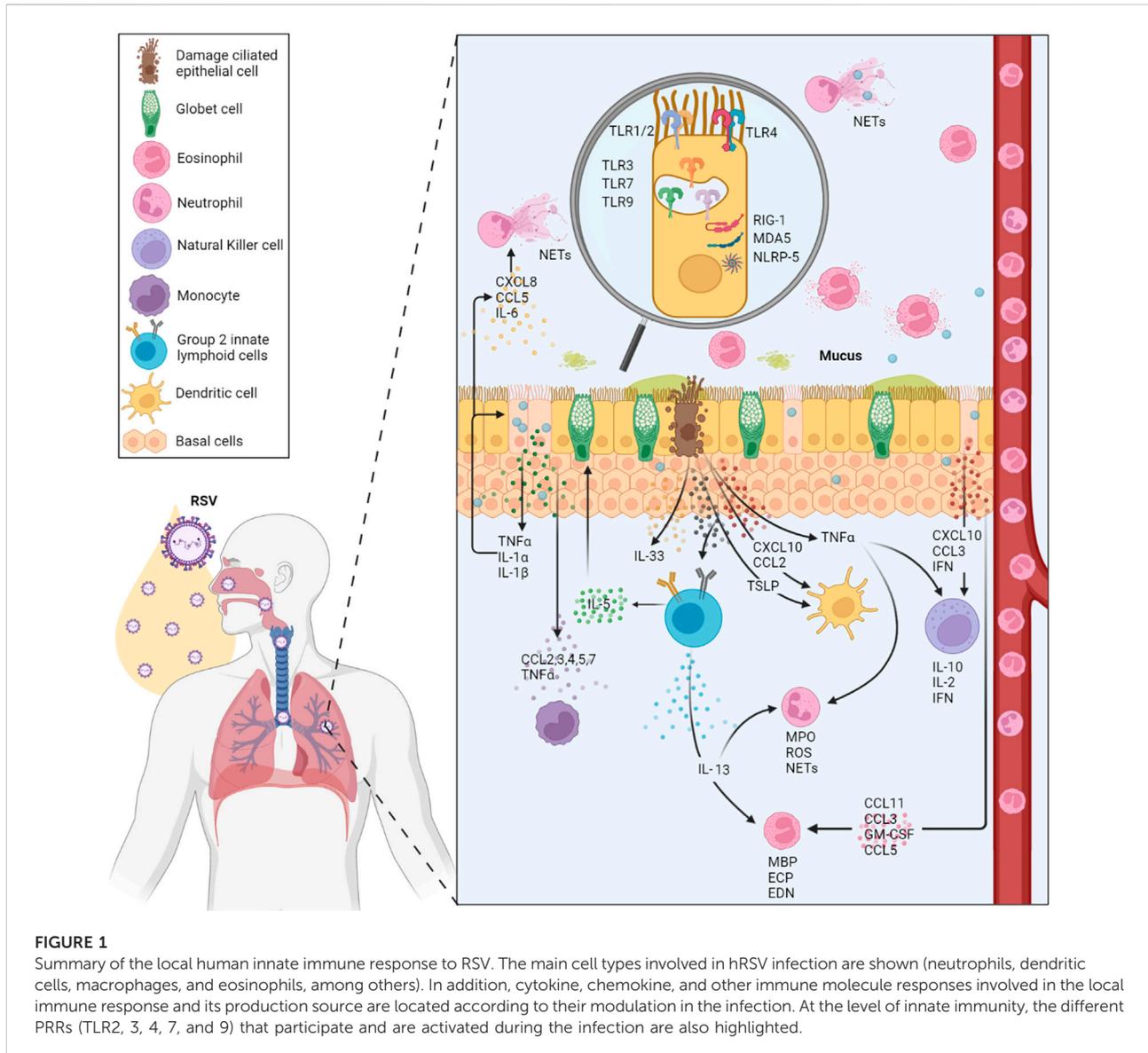
2005). The Toll-like receptor 4 (TLR4) binds the F protein expressed on the ciliated bronchial (Marzec et al., 2019) and epithelial cells (Park et al., 2012), triggering innate immune signaling during the hRSV entry (Funchal et al., 2015). However, TLR4 activates kinases to potentiate viral entry through endocytosis (Walsh et al., 1997; Piedra et al., 2003). Another potential receptor is the CX3 chemokine receptor 1 (CX3CR1) (Anderson et al., 2020), which binds the G protein on the apical side of ciliated bronchial epithelial cells (Anderson et al., 2021). Mice deficient in CX3CR1 are less susceptible to hRSV infection, as RSV-G and CX3CR1 interaction could alter chemotaxis signaling (Anderson et al., 2020; Anderson et al., 2021). Other reports have described nucleolin binding to the F protein (Hall et al., 1986; Nielsen et al., 2003). As nucleolin is highly expressed on the surface of dividing cells, it could be pivotal in children's lower respiratory tract infections (Parrott et al., 1973; Imaz et al., 2000; Roca et al., 2002; Ochola et al., 2009).

## Innate immune response to hRSV infection

Several factors are involved in the innate immune response against hRSV infection; airway epithelial cells, dendritic cells, macrophages, monocytes, granulocytes, as well as pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and NOD-like receptors (NLRs) (Yamaguchi et al., 2011; Zeng et al., 2012) (Figure 1).

Airway epithelial cells recognize viral PAMPs through different PRRs, initiating early immune response (Lay et al., 2013). This activation occurred via MyD88 and TRIF (Kumar et al., 2011), and then, different transcription factors such as interferon-regulatory factor 3 (IRF-3), nuclear factor  $\kappa$ B (NF- $\kappa$ B) and ATF-2/cJun are activated. These factors mainly promote the transcription of antiviral genes, dendritic cells activation, and the production of soluble molecules such as pro-inflammatory cytokines and chemokines by dendritic cells and alveolar macrophages (Goritzka et al., 2015; Lay et al., 2016; Feng et al., 2018).

Different TLRs have been associated with hRSV infection: TLR4 interacts with hRSV F protein using CD14 as a co-receptor leading to NF- $\kappa$ B activation and mediated innate immune responses and inflammation (Kurt-Jones et al., 2000). Recently, it was demonstrated that RSV-induced oxidative stress promotes enhanced activation and release of Transglutaminase 2 from human lung epithelial cells, which is mediated by Toll-like receptor (TLR)-4 and NF- $\kappa$ B pathways. Transglutaminase 2 is an enzyme implicated in various pathological conditions, but its role in hRSV remains unclear (Rayavara et al., 2022). TLR-2 regulates pro-IL-1 $\beta$  and NLRP3 gene expression during RSV infection. The TLR2 activation is the first signal necessary for the posterior



formation of the NLRP3 inflammasome, leading to caspase-1 activation and subsequent IL-1 $\beta$  release during RSV infection (Segovia et al., 2012).

On endosomal compartments, the recognition of the genome (ssRNA) and replication intermediaries (dsRNA) occurs through TLR7 and TLR3, respectively (Aeffner et al., 2011). hRSV infection triggers the activation of the TLR3 signaling pathways that regulate the expression of MyD88-independent chemokines, such as IP-10/CXCL10 and CCL5, and further upregulate TLR3 expression in RSV-infected cells (Rudd et al., 2005). Also, it has been demonstrated that activation of TLR3 during hRSV infection promotes a predominantly Th1-type response, contributing to the establishment of the adaptive immune response (Rudd et al., 2006). TLR7 plays a crucial role in RSV detection and subsequent response immune initiation. RSV

is recognized by classical and plasmacytoid DCs through TLR7, inducing the anti-RSV response and immunomodulatory effects (Smit et al., 2006; Lukacs et al., 2010). A study revealed that TLR7 regulates IL-12/IL-23 responsiveness to RSV in dendritic cells and demonstrated that TLR7  $-/-$  bone marrow-derived dendritic cells were significantly impaired in the induction of IL-12 in response to RSV but exhibited significantly higher production of IL-23 (Lindell et al., 2009). In addition, dendritic cells are sources of interferon- $\beta$  in RSV, amplifying early antiviral responses (Kim et al., 2019).

In response to hRSV infection, the airway epithelial cells produce pro-inflammatory cytokines such as type-I and type-III interferons (IFN) that bind to its receptors (IFNRs) and activate signaling pathways through the Signal Transducer and Activator of Transcription 1 and 2 (STAT-1 and STAT-2). STAT binds to

**TABLE 1** Function of immune molecules during hRSV infection.

Molecules	Response
CX3CR1	Interacts with viral G-protein to allow viral entry
MyD88 and TRIF	Early immune response and activation of transcription factors
IRF-3, NF- $\kappa$ B and ATF-2/cJun	Promote transcription of antiviral genes, DCs activation and pro-inflammatory cytokines production
TLR2	Regulates pro-IL-1b and NLRP3 gene expression
TLR3	Intermediates viral replication in endosomes, regulates the expression of MyD88 and lead a Th1-response profile
TLR4 and CD14	Bind to viral F-protein
TLR7	Recognition of viral genome in endosomes and regulates IL-12/IL-23 responsiveness
NLRP3	Lead to caspase-1 activation and subsequent IL-1b release
IFN-II and IFN-III	activate signaling pathways through STAT-1 and STAT-2
STAT	Binds to IFN-regulatory factors in the ISGs
CCL2 and CCL5	Promote the recruitment of monocytes, neutrophils, dendritic cells, macrophages, natural killer cells, and CD4 <sup>+</sup> T cells to the site of infection

CX3CR1, C-X3-C motif chemokine receptor 1; IRF-3, interferon-regulatory factor 3; IFN-II and -III, type-II and type-III interferons; NF- $\kappa$ B, nuclear factor  $\kappa$ B; TLR, Toll-like receptors; STAT, Signal Transducer and Activator of Transcription; ISGs, Interferon-stimulated genes.

IFN-regulatory factors in the interferon-stimulated genes (ISGs). Finally, several pro-inflammatory cytokines such as IL-6, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and chemokines (CXCL8, CCL3, CCL2, and CCL5) are induced and secreted. Some chemokines, such as CCL2 and CCL5, could promote the recruitment of monocytes, neutrophils, dendritic cells, macrophages, natural killer cells, and CD4<sup>+</sup> T cells to the site of infection (Lay et al., 2016; Aronen et al., 2019) (Table 1).

*In vitro*, airway epithelial cells produce IL-1 $\beta$ , IL-6, and TNF $\alpha$ , macrophage inflammatory protein-1a (MIP-1a/CCL3), monocyte chemoattractant protein-1 (MCP-1/CCL2), RANTES (regulated on activation, normally T cell-expressed and secreted/CCL5), eotaxin (CCL11), IL-8 (CXCL8), monokine induced by IFN $\gamma$  (MIG/CXCL9), IP-10 (CXCL10), and fractalkine (CX3CL1) (Bonville et al., 1999; Miller et al., 2004; Mochizuki et al., 2009; Villenave et al., 2011). The effect of this cytokine production during hRSV infection is still controversial (Tayyari et al., 2011; Villenave et al., 2012). It has been proposed that cell lines, compared with primary cells, induced different cytokine profiles, even when exposed to the same strain of the virus (Fonceca et al., 2012). In the same way, cells from different donors significantly alter the profile. Besides, the localization of the epithelial cells in the airways seems to affect the constitutive production and cytokines upregulation (Olszewska-Pazdrak et al., 1998).

During the inflammatory process caused by the hRSV infection in the airways of infants with bronchiolitis,

prominent infiltration of neutrophils is observed (Habibi et al., 2020). During severe infection, the virus interacts directly with neutrophils; these cells in BAL and the blood of infants with severe RSV infection expressed RSV N genomic RNA, indicating uptake of the whole virus (Halfhide et al., 2011). In response, neutrophils secrete cytokines, toxic proteins, and granular enzymes, including myeloperoxidase (MPO), elastase and defensins. Furthermore, ROS are released to the extracellular environment in response to viral infections. Neutrophil extracellular trap (NET) formation is active during infection, and NETs are present in BAL fluid from ventilated children. Furthermore, NETs captured RSV, precluding viral particles' binding to target cells and preventing infection. However, excessive NETs formation contributes to the immunopathology developed by patients infected with hRSV (Cortjens et al., 2016).

In addition, superoxide production has also been observed in eosinophils after hRSV exposure. Additionally, it was observed that eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) reduced the infectivity of hRSV after exposure, suggesting their antiviral activity. On the other hand, it has been demonstrated that myelin basic protein (MBP) promotes the cell death of hRSV-infected epithelial cells (Glaser et al., 2019).

## Viral evasion mechanisms

Different mechanisms have been associated with poor immune control. An optimal clearance of the hRSV requires a Th1 and Th2 balance, which promotes IFN $\gamma$  production by cytotoxic CD8<sup>+</sup> T cells (Schmidt et al., 2018). Besides, hRSV infection does not seem to engage an effective memory response that protects from future viral exposures. During convalescence, circulating RSV IgG- but not IgA-producing memory B cells are present; this deficit IgA memory may contribute to recurrent infections, especially in childhood (Russell et al., 2017).

hRSV impairs the assembly of a proper immunological synapse between the antigen-presenting cells (APC), such as the dendritic cells and T cells. The virus renders T cells unable to respond correctly, affecting the adaptive immune response and increasing the risk of reinfections (Gonzalez et al., 2008). The hRSV has been observed to infect dendritic cells and impair their maturation without affecting their ability to present antigens and prime T cells. However, the infection alters the cytokine milieu by inhibiting the formation of the immune synapse, producing inhibitory factors and affecting the viral clearance through the NS1 and NS2 proteins that hamper IFNs production (Collins and Graham, 2008; van Drunen Littel-van den Hurk and Watkiss, 2012). It is well known that an immature or hypofunctional immune system facilitates infection with RSV in neonates and young infants. In this sense, macrophages from neonatal individuals exhibit a diminished expression of PRRs or a

reduced upregulation upon activation with a belittled cytokine production (Basha et al., 2014).

A limited antigenic diversity is observed in hRSV strains compared to other respiratory viruses. However, reinfections with hRSV occur throughout life, and this can result from an incomplete and short-lived protective immunity. Nonetheless, hRSV has developed several mechanisms of evasion which could explain the impairing of the immune response (van Drunen Littel-van den Hurk and Watkiss, 2012). The G and F proteins are heavily glycosylated antigens that produce neutralizing antibodies, which could interfere with antibody recognition (García-Beato and Melero, 2000; Palomo et al., 2000). In the central conserved domain of the G protein, a CX3C motif resembles the one in the chemokine fractalkine; this motif shows *in vitro* a similar leukocyte chemotactic activity (Jarti et al., 2009). It impairs the activation of the NF- $\kappa$ B and the production of inflammatory cytokines in human monocytes, suggesting an immune inhibitory role (Levine et al., 2004). It has been described that the secreted form of G protein inhibits TLR3/4 mediated IFN-beta *in vitro* (Shingai et al., 2008), probably suppressing the innate immune response.

The F protein responsible for the fusion and subsequent syncytia formation (Sun et al., 2013) is critical in the viral attachment, interacting with the hRSV receptor: nucleolin (Resch et al., 2007). This protein induces the innate immune response through TLR4 on leukocytes (Freymuth et al., 1997) and epithelial cells, facilitating p53-dependent apoptosis (Freymuth et al., 2006). The SH protein has been implicated in inhibiting apoptosis by the TNF- $\alpha$  pathway (Stockman et al., 2012; Zhou et al., 2012).

On the other hand, the production of G protein occurred in a full-length membrane-bound form and truncated secreted form. The secreted protein seems to decoy the neutralizing antibodies (van Drunen Littel-van den Hurk and Watkiss, 2012). Besides, its CX3C motif allows the signaling through the CX3CR1 receptor and confers a chemotactic activity similar to fractalkine (CX3CL1). It is unclear if this capacity affects the leukocyte recruitment to the infected lungs *in vivo* (Collins and Graham, 2008). Some studies have reported how the G protein can induce specific T cell clones to produce IL-4 and IL-10, while the T cells specific to the F protein induce a Th1 immune response that mimics the answer raised by whole hRSV (Jackson and Scott, 1996). These data suggest that the G protein could downregulate cellular immunity and induce a negative immune regulation, antagonizing TLRs signaling (Collins and Graham, 2008; van Drunen Littel-van den Hurk and Watkiss, 2012).

## TH1-TH2 immune response in hRSV infection

Variable results have been obtained when children's cytokine profiles are analyzed in primary hRSV infection. Some studies found no change or decreased IFN $\gamma$  levels in mitogen-stimulated

peripheral blood mononuclear cells (PBMC) (Bendelja et al., 2000; Pinto et al., 2006). This cytokine's production was also low compared with other respiratory infection agents (Aberle et al., 2004).

Some conflicting results around the concurrent upregulation of Th2 cytokines suggest a Th2 dominance (Bendelja et al., 2000; Gut et al., 2013), while other researchers could not find an IL-4 or IL-5 production (Pinto et al., 2006). Besides, the specific role of IFN $\gamma$  production by PBMC and the positive correlation with disease severity appears to be difficult to establish even when other data propose a protective effect for this cytokine (Bendelja et al., 2000). A study suggests a combined Th1 and Th2 immune response, RSV stimulation of PBMC from RSV-hospitalized patients results in Th1 and Th2 cytokine expression, accompanied by enhanced production of IL-2, IL-4, and IL-13 (Tripp et al., 2002). Another study reported a predominant production of IFN-gamma and low levels of IL-4 and IL-10, but any association of clinical severity with T cell profile was not observed (Brandenburg et al., 2000).

Although hRSV RNA has been detected in blood monocytes and neutrophils, it is well known that the hRSV infection is restricted to the respiratory tract (O'Donnell et al., 1998; Halfhide et al., 2011). Consequently, the analysis of PBMC could not reflect the local immune responses occurring in the respiratory tract; besides, hRSV-specific CD8<sup>+</sup> T cells are identified and show quantitative differences between blood and the respiratory location, indicating active recruitment into the tissue (Heidema et al., 2008). Compared with the lower respiratory tract, cytokine levels in the upper respiratory tract have a strong or moderate correlation in children with ventilation (van Schaik et al., 1999; Semple et al., 2007). However, these data are controversial; increased IFN $\gamma$  production is observed during the acute hRSV infection compared to healthy controls (Garofalo et al., 2001; Kim et al., 2012) or bronchiolitis patients unrelated to hRSV (Bennett et al., 2007). In contrast, in other studies, a low or undetectable IFN $\gamma$  production has been reported (Kristjansson et al., 2005).

In the same way, although increased levels of Th2 cytokines have been described (Legg et al., 2003), other data report low or undetectable levels, similar to controls (Bont et al., 2001; Pinto et al., 2006; Kim et al., 2012). The Th2-skewing has been found in patients with ambulatory lower respiratory tract infection compared to those with upper respiratory tract infection (Legg et al., 2003). However, other studies propose a Th2-skewing in upper respiratory tract infection and hypoxic bronchiolitis (Garofalo et al., 2001). Virus-induced wheezing is characterized by an immunologic imbalance, resulting in excessive release of IFN- $\gamma$  in the airway of patients with bronchiolitis (van Schaik et al., 1999). A significant inter-individual disparity in the expression of Th1 and Th2 cytokines and the dominant cytokine profile has been found in cytokine mRNA response analysis (Mobbs et al., 2002). Another study evaluated nasopharyngeal secretions in

infected infants, reporting that hRSV infection promotes Th2-like response in the nose with local production of IL-4, IL-5, macrophage inflammatory protein 1b, and infiltration and activation of eosinophils (Kristjansson et al., 2005).

A noticeable age-dependence has been observed in IFN $\gamma$  and IL-12 production with the responsiveness to IL-12 (type 1 immune responses inductor) (Krampera et al., 1999; Buck et al., 2002; Motegi et al., 2006; Yerkovich et al., 2007). IL-12 and IL-18 have independent effects on their role in the induction of IFN $\gamma$  production, and it seems to protect from bronchiolitis (van Benten et al., 2003). It is unclear if the higher IL-4 production and lower IFN $\gamma$  production are relevant in the hRSV infections, which seem to be related to the age in mice models (Culley et al., 2002; Dakhama et al., 2005). In the same way, the disease severity impacts on the lower respiratory tract after the first infection and the immune response in secondary infections remains unclear. The IL-10 levels in different specimens are higher in some cohorts (Sheeran et al., 1999; Chung et al., 2005) and similar in others (van Schaik et al., 1999; Joshi et al., 2003; Pinto et al., 2006). During acute infection, some studies suggest a link between marked IL-10 production and atopy (Chung et al., 2005). However, other reports deny this association (Joshi et al., 2003).

Moreover, the nasopharyngeal or tracheal IL-10 levels strongly correlate with severity (Sheeran et al., 1999; Bennett et al., 2007; Vieira et al., 2010). The pro-inflammatory and regulatory profile of IL-10 could be partially responsible for the discrepancies that the researchers report. Besides, the timing and intensity of production may influence its role as a protective or detrimental factor. On the other hand, methodological issues and the differential capacity to induce IL-10 that the specific strains of hRSV exhibit could influence the results (Lukacs et al., 2006).

Independently of Th1/Th2 response, robust production of IL-13 has been observed during the early phase of RSV infection in a murine model. This production of IL-13 in several other pulmonary diseases is mediated for Group 2 innate lymphoid cells and can contribute to immunopathology during RSV infection (Stier et al., 2016).

## Immunity in animal models

In the search for treatments and vaccines, animal models have been necessary to understand the immune response against hRSV. Still, no animal can widely represent the immune response in humans, so it has been necessary to use different animal models (Taylor, 2017). Several animals have been used as models, including cotton rats, mice, ferrets, guinea pigs, hamsters, chinchillas, neonatal lambs, bovines, and non-human primates like chimpanzees, African green monkeys, and macaques (Taylor, 2017).

Replicating the hRSV infection in animals is difficult because these are not entirely permissive to the virus, and clinical signs

are not present in some cases; chimpanzees are the only non-human primate permissive to the virus with symptom development on the respiratory tract (Teng et al., 2000). Green monkeys are less permissive to the virus than chimpanzees, but the viral load is a reliable parameter to determine in this model (Ispas et al., 2015).

Despite differences between humans and animals, the mice model is one of the most used for testing vaccine development and elucidation of immunopathogenic mechanisms in the hRSV infection context (Mazur et al., 2018; Andersen and Winter, 2019). For example, the Th2 immune response polarization has been observed in this model, where Th2 cytokines (IL-4, IL-10, IL-13, and CCL5) were upregulated (Sawada and Nakayama, 2016).

The use of animal models exhibits great benefits in understanding the pathology of the infection and is also essential for preclinical studies to achieve vaccine development. In the same way, different studies emphasize that there is no reliable animal model to approximate the morbidity and mortality of hRSV infection in humans. For this reason, the study of infected humans is very important (Altamirano-Lagos et al., 2019).

## Current information on vaccines against hRSV

Target populations for hRSV vaccination include infants, pregnant women, and older adults. Despite there being no approved vaccines against hRSV for all target populations available, there are several candidates in different clinical trial phases. Currently, scientists have dedicated their efforts to 33 vaccine candidates using 6 hRSV vaccine platforms, 9 candidates are in pivotal phase III clinical trials, and 2 are approved by US Food and Drug Administration (FDA). These platforms are particle-based vaccines, vector-based vaccines, live-attenuated viral particles or chimeric vaccines, subunit vaccines, mRNA vaccines, and monoclonal antibodies (Qiu et al., 2022; Mazur et al., 2023).

There are three target populations: pediatric, maternal and older adults. For pediatrics, strategies include passive immunoprophylaxis with monoclonal antibodies, live-attenuated vaccines and passive immunization with maternal antibodies; for maternal subunit vaccines are in late-stage studies to protect infants and; for older adults, strategies include vector, subunit, and nucleic acid approaches (Mazur et al., 2023).

GSK's landmark positive pivotal AReSVi-006 (Adult Respiratory Syncytial Virus) phase III trial data showed that adults aged 60 years or older receiving a single dose of an AS01E-adjuvanted RSV prefusion F protein-based vaccine (RSVPreF3 OA) exhibited an efficacy against hRSV of 82.6% (96.95% confidence interval [CI], 57.9–94.1) (Papi et al., 2023), making this, in May 2023, the first hRSV vaccine approved by

FDA to be used in adults aged 60 years or older (U.S. Food and Drug Administration, 2023). Recently, the FDA has approved the Pfizer vaccine ABRISVOTM for older adults, a RSV A and RSV B prefusion F protein, employed in the RENOIR phase 3 clinical trial (NCT05035212) which enrolled 35,971 participants and exhibited a vaccine efficacy of 85.7% (96.66% confidence interval [CI], 32.0–98.7) (Walsh et al., 2023). These current vaccines are the result of years of research, and have the potential to reduce the incidence, morbidity, mortality, and economic burden of hRSV infections worldwide.

## Conclusion

The innate immune response has a crucial role during hRSV infection. Its modulation has been widely demonstrated by hRSV in which activation of PRRs, induction of pro-inflammatory cytokines, induction of antiviral response, and shape of adaptive immune response have been described. Local and systemic immune responses induced during viral infection have been associated with pathogenesis, and the contribution of Th1 and Th2 immune responses is variable. Understanding the immune response generated during hRSV infection is necessary for developing new therapeutics that can modulate the immunopathogenesis of hRSV infection. These advances would also be valuable for developing an effective and safe

vaccine necessary for all target populations and ameliorate the burden of RSV infection in public health.

## Author contributions

DC, DMG and SG: Writing—Original Draft. NAT and JCH: Conceptualization; Writing—Review & Editing. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

## Funding

This work was supported by Universidad Cooperativa de Colombia and Corporación Universitaria Remington. The funders had no role in the design of the study, data collection and analysis, decision to publish, or preparation of the manuscript.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References

- Aberle, J. H., Aberle, S. W., Rebhandl, W., Pracher, E., Kundi, M., and Popow-Kraupp, T. (2004). Decreased interferon-gamma response in respiratory syncytial virus compared to other respiratory viral infections in infants. *Clin. Exp. Immunol.* 137, 146–150. doi:10.1111/j.1365-2249.2004.02504.x
- Aeffner, F., Traylor, Z. P., Yu, E. N., and Davis, I. C. (2011). Double-stranded RNA induces similar pulmonary dysfunction to respiratory syncytial virus in BALB/c mice. *Am. J. Physiol. Lung Cell Mol. Physiol.* 301, L99–L109. doi:10.1152/ajplung.00398.2010
- Afonso, C. L., Amarasinghe, G. K., Bányai, K., Bào, Y., Basler, C. F., Bavari, S., et al. (2016). Taxonomy of the order mononegavirales: update 2016. *Arch. Virol.* 161, 2351–2360. doi:10.1007/s00705-016-2880-1
- Altamirano-Lagos, M. J., Diaz, F. E., Mansilla, M. A., Rivera-Perez, D., Soto, D., McGill, J. L., et al. (2019). Current animal models for understanding the pathology caused by the respiratory syncytial virus. *Front. Microbiol.* 10, 873. doi:10.3389/fmicb.2019.00873
- Andersen, M. L., and Winter, L. M. F. (2019). Animal models in biological and biomedical research - experimental and ethical concerns. *Acad Bras Cienc* 91, e20170238. doi:10.1590/0001-3765201720170238
- Anderson, C. S., Chu, C. Y., Wang, Q., Mereness, J. A., Ren, Y., Donlon, K., et al. (2020). CX3CR1 as a respiratory syncytial virus receptor in pediatric human lung. *Pediatr. Res.* 87, 862–867. doi:10.1038/s41390-019-0677-0
- Anderson, C. S., Chirkova, T., Slaunwhite, C. G., Qiu, X., Walsh, E. E., Anderson, L. J., et al. (2021). CX3CR1 engagement by respiratory syncytial virus leads to induction of nucleolin and dysregulation of cilia-related genes. *J. Virol.* 95, e00095-21. doi:10.1128/JVI.00095-21
- Aronen, M., Viikari, L., Kohonen, I., Vuorinen, T., Hameenaho, M., Wuorela, M., et al. (2019). Respiratory tract virus infections in the elderly with pneumonia. *BMC Geriatr.* 19, 111. doi:10.1186/s12877-019-1125-z
- Barr, R., Green, C. A., Sande, C. J., and Drysdale, S. B. (2019). Respiratory syncytial virus: diagnosis, prevention and management. *Ther. Adv. Infect. Dis.* 6, 204993611986579. doi:10.1177/2049936119865798
- Basha, S., Surendran, N., and Pichichero, M. (2014). Immune responses in neonates. *Expert Rev. Clin. Immunol.* 10, 1171–1184. doi:10.1586/1744666X.2014.942288
- Battles, M. B., and Mclellan, J. S. (2019). Respiratory syncytial virus entry and how to block it. *Nat. Rev. Microbiol.* 17, 233–245. doi:10.1038/s41579-019-0149-x
- Bendelja, K., Gagro, A., Bace, A., Lokar-Kolbas, R., Krsulovic-Hresic, V., Drazenovic, V., et al. (2000). Predominant type-2 response in infants with respiratory syncytial virus (RSV) infection demonstrated by cytokine flow cytometry. *Clin. Exp. Immunol.* 121, 332–338. doi:10.1046/j.1365-2249.2000.01297.x
- Bennett, B. L., Garofalo, R. P., Cron, S. G., Hosakote, Y. M., Atmar, R. L., Macias, C. G., et al. (2007). Immunopathogenesis of respiratory syncytial virus bronchiolitis. *J. Infect. Dis.* 195, 1532–1540. doi:10.1086/515575
- Bont, L., Heijnen, C. J., Kavelaars, A., Van Aalderen, W. M., Brus, F., Draaisma, J. M., et al. (2001). Local interferon-gamma levels during respiratory syncytial virus lower respiratory tract infection are associated with disease severity. *J. Infect. Dis.* 184, 355–358. doi:10.1086/322035
- Bonville, C. A., Rosenberg, H. F., and Domachowski, J. B. (1999). Macrophage inflammatory protein-1 $\alpha$  and RANTES are present in nasal secretions during ongoing upper respiratory tract infection: chemokines induced by URIs. *Pediatr. Allergy Immunol.* 10, 39–44. doi:10.1034/j.1399-3038.1999.101005.x
- Botosso, V. F., Zanutto, P. M., Ueda, M., Arruda, E., Gilio, A. E., Vieira, S. E., et al. (2009). Positive selection results in frequent reversible amino acid replacements in the G protein gene of human respiratory syncytial virus. *PLoS Pathog.* 5, e1000254. doi:10.1371/journal.ppat.1000254
- Bradley, J. P., Bacharier, L. B., Bonfiglio, J., Schechtman, K. B., Strunk, R., Storch, G., et al. (2005). Severity of respiratory syncytial virus bronchiolitis is affected by cigarette smoke exposure and atopy. *Pediatrics* 115, e7–e14. doi:10.1542/peds.2004-0059
- Brandenburg, A. H., Kleinjan, A., Van Het Land, B., Moll, H. A., Timmerman, H. H., De Swart, R. L., et al. (2000). Type 1-like immune response is found in children

- with respiratory syncytial virus infection regardless of clinical severity. *J. Med. Virol.* 62, 267–277. doi:10.1002/1096-9071(200010)62:2<267:AID-JMV20>3.0.CO;2-8
- Braun, M. R., Noton, S. L., Blanchard, E. L., Shareef, A., Santangelo, P. J., Johnson, W. E., et al. (2021). Respiratory syncytial virus M2-1 protein associates non-specifically with viral messenger RNA and with specific cellular messenger RNA transcripts. *PLoS Pathog.* 17, e1009589. doi:10.1371/journal.ppat.1009589
- Buck, R. H., Cordle, C. T., Thomas, D. J., Winship, T. R., Schaller, J. P., and Dugle, J. E. (2002). Longitudinal study of intracellular T cell cytokine production in infants compared to adults. *Clin. Exp. Immunol.* 128, 490–497. doi:10.1046/j.1365-2249.2002.01851.x
- Cao, D., Gao, Y., and Liang, B. (2021). Structural insights into the respiratory syncytial virus RNA synthesis complexes. *Viruses* 13, 834. doi:10.3390/v13050834
- Checchia, P. A., Paes, B., Bont, L., Manzoni, P., Simões, E. A., Fauroux, B., et al. (2017). Defining the risk and associated morbidity and mortality of severe respiratory syncytial virus infection among infants with congenital heart disease. *Infect. Dis. Ther.* 6, 37–56. doi:10.1007/s40121-016-0142-x
- Chung, H. L., Kim, W. T., Kim, J. K., Choi, E. J., Lee, J. H., Lee, G. H., et al. (2005). Relationship between atopic status and nasal interleukin 10 and 11 levels in infants with respiratory syncytial virus bronchiolitis. *Ann. Allergy Asthma Immunol.* 94, 267–272. doi:10.1016/S1081-1206(10)61307-5
- Collins, P. L., and Graham, B. S. (2008). Viral and host factors in human respiratory syncytial virus pathogenesis. *J. Virol.* 82, 2040–2055. doi:10.1128/JVI.01625-07
- Cortjens, B., De Boer, O. J., De Jong, R., Antonis, A. F., Sabogal Pineros, Y. S., Lutter, R., et al. (2016). Neutrophil extracellular traps cause airway obstruction during respiratory syncytial virus disease. *J. Pathol.* 238, 401–411. doi:10.1002/path.4660
- Culley, F. J., Pollott, J., and Openshaw, P. J. (2002). Age at first viral infection determines the pattern of T cell-mediated disease during reinfection in adulthood. *J. Exp. Med.* 196, 1381–1386. doi:10.1084/jem.20020943
- Dakhama, A., Park, J. W., Taube, C., Joetham, A., Balhorn, A., Miyahara, N., et al. (2005). The enhancement or prevention of airway hyperresponsiveness during reinfection with respiratory syncytial virus is critically dependent on the age at first infection and IL-13 production. *J. Immunol.* 175, 1876–1883. doi:10.4049/jimmunol.175.3.1876
- Devincenzo, J. P. (2004). Natural infection of infants with respiratory syncytial virus subgroups A and B: a study of frequency, disease severity, and viral load. *Pediatr. Res.* 56, 914–917. doi:10.1203/01.PDR.0000145255.86117.6A
- Falsey, A. R., Hennessey, P. A., Formica, M. A., Cox, C., and Walsh, E. E. (2005). Respiratory syncytial virus infection in elderly and high-risk adults. *N. Engl. J. Med.* 352, 1749–1759. doi:10.1056/NEJMoa043951
- Feng, S., Zeng, D., Zheng, J., and Zhao, D. (2018). MicroRNAs: mediators and therapeutic targets to airway hyper reactivity after respiratory syncytial virus infection. *Front. Microbiol.* 9, 2177. doi:10.3389/fmicb.2018.02177
- Fjaerli, H. O., Farstad, T., and Bratlid, D. (2004). Hospitalisations for respiratory syncytial virus bronchiolitis in akershus, Norway, 1993-2000: a population-based retrospective study. *BMC Pediatr.* 4, 25. doi:10.1186/1471-2431-4-25
- Fonceca, A. M., Flanagan, B. F., Trinick, R., Smyth, R. L., and Mcnamara, P. S. (2012). Primary airway epithelial cultures from children are highly permissive to respiratory syncytial virus infection. *Thorax* 67, 42–48. doi:10.1136/thoraxjnl-2011-200131
- Freymuth, F., Vabret, A., Galateau-Salle, F., Ferey, J., Eugene, G., Petitjean, J., et al. (1997). Detection of respiratory syncytial virus, parainfluenzavirus 3, adenovirus and rhinovirus sequences in respiratory tract of infants by polymerase chain reaction and hybridization. *Clin. Diagn. Virol.* 8, 31–40. doi:10.1016/s0928-0197(97)00060-3
- Freymuth, F., Vabret, A., Cuvillon-Nimal, D., Simon, S., Dina, J., Legrand, L., et al. (2006). Comparison of multiplex PCR assays and conventional techniques for the diagnostic of respiratory virus infections in children admitted to hospital with an acute respiratory illness. *J. Med. Virol.* 78, 1498–1504. doi:10.1002/jmv.20725
- Funchal, G. A., Jaeger, N., Czepielewski, R. S., Machado, M. S., Muraro, S. P., Stein, R. T., et al. (2015). Respiratory syncytial virus fusion protein promotes TLR-4-dependent neutrophil extracellular trap formation by human neutrophils. *PLoS One* 10, e0124082. doi:10.1371/journal.pone.0124082
- García-Beato, R., and Melero, J. A. (2000). The C-terminal third of human respiratory syncytial virus attachment (G) protein is partially resistant to protease digestion and is glycosylated in a cell-type-specific manner. *J. Gen. Virol.* 81, 919–927. doi:10.1099/0022-1317-81-4-919
- Garofalo, R. P., Patti, J., Hintz, K. A., Hill, V., Ogra, P. L., and Welliver, R. C. (2001). Macrophage inflammatory protein-1 $\alpha$  (not T helper type 2 cytokines) is associated with severe forms of respiratory syncytial virus bronchiolitis. *J. Infect. Dis.* 184, 393–399. doi:10.1086/322788
- Gelfand, E. W. (2012). Development of asthma is determined by the age-dependent host response to respiratory virus infection: therapeutic implications. *Curr. Opin. Immunol.* 24, 713–719. doi:10.1016/j.coi.2012.08.011
- Glaser, L., Coulter, P. J., Shields, M., Touzelet, O., Power, U. F., and Broadbent, L. (2019). Airway epithelial derived cytokines and chemokines and their role in the immune response to respiratory syncytial virus infection. *Pathogens* 8, 106. doi:10.3390/pathogens8030106
- Gonzalez, P. A., Prado, C. E., Leiva, E. D., Carreno, L. J., Bueno, S. M., Riedel, C. A., et al. (2008). Respiratory syncytial virus impairs T cell activation by preventing synapse assembly with dendritic cells. *Proc. Natl. Acad. Sci. U. S. A.* 105, 14999–15004. doi:10.1073/pnas.0802555105
- Grortzka, M., Makris, S., Kausar, F., Durant, L. R., Pereira, C., Kumagai, Y., et al. (2015). Alveolar macrophage-derived type I interferons orchestrate innate immunity to RSV through recruitment of antiviral monocytes. *J. Exp. Med.* 212, 699–714. doi:10.1084/jem.20140825
- Griffiths, C., Drews, S. J., and Marchant, D. J. (2017). Respiratory syncytial virus: infection, detection, and new options for prevention and treatment. *Clin. Microbiol. Rev.* 30, 277–319. doi:10.1128/CMR.00010-16
- Gut, W., Pancer, K., Abramczuk, E., Czescik, A., Dunal-Szczepaniak, M., Lipka, B., et al. (2013). RSV respiratory infection in children under 5 y. o. -dynamics of the immune response Th1/Th2 and IgE. *Przegl. Epidemiol.* 67, 17–22. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/23745370> (Accessed November 23, 2022).
- Habibi, M. S., Thwaites, R. S., Chang, M., Jozwik, A., Paras, A., Kirsebom, F., et al. (2020). Neutrophilic inflammation in the respiratory mucosa predisposes to RSV infection. *Science* 370, eaba9301. doi:10.1126/science.aba9301
- Halfhide, C. P., Flanagan, B. F., Brearey, S. P., Hunt, J. A., Fonceca, A. M., Mcnamara, P. S., et al. (2011). Respiratory syncytial virus binds and undergoes transcription in neutrophils from the blood and airways of infants with severe bronchiolitis. *J. Infect. Dis.* 204, 451–458. doi:10.1093/infdis/jir280
- Hall, C. B., Powell, K. R., Macdonald, N. E., Gala, C. L., Menegus, M. E., Suffin, S. C., et al. (1986). Respiratory syncytial viral infection in children with compromised immune function. *N. Engl. J. Med.* 315, 77–81. doi:10.1056/NEJM198607103150201
- Hall, C. B. (2001). Respiratory syncytial virus and parainfluenza virus. *N. Engl. J. Med.* 344, 1917–1928. doi:10.1056/NEJM200106213442507
- Heidema, J., Rossen, J. W., Lukens, M. V., Ketel, M. S., Scheltens, E., Kranendonk, M. E., et al. (2008). Dynamics of human respiratory virus-specific CD8+ T cell responses in blood and airways during episodes of common cold. *J. Immunol.* 181, 5551–5559. doi:10.4049/jimmunol.181.8.5551
- Hu, P., Zheng, T., Chen, J., Zhou, T., Chen, Y., Xu, X., et al. (2017). Alternate circulation and genetic variation of human respiratory syncytial virus genotypes in Chengdu, West China, 2009-2014. *J. Med. Virol.* 89, 32–40. doi:10.1002/jmv.24603
- Imaz, M. S., Sequeira, M. D., Videla, C., Veronessi, I., Cociglio, R., Zerbini, E., et al. (2000). Clinical and epidemiologic characteristics of respiratory syncytial virus subgroups A and B infections in Santa Fe, Argentina. *J. Med. Virol.* 61, 76–80. doi:10.1002/(sici)1096-9071(200005)61:1<76:aid-jmv12>3.0.co;2-p
- Ispas, G., Koul, A., Verbeeck, J., Sheehan, J., Sanders-Ber, B., Roymans, D., et al. (2015). Antiviral activity of TMC353121, a respiratory syncytial virus (RSV) fusion inhibitor, in a non-human primate model. *PLoS One* 10, e0126959. doi:10.1371/journal.pone.0126959
- Jackson, M., and Scott, R. (1996). Different patterns of cytokine induction in cultures of respiratory syncytial (RS) virus-specific human T<sub>H</sub> cell lines following stimulation with RS virus and RS virus proteins. *J. Med. Virol.* 49, 161–169. doi:10.1002/(SICI)1096-9071(199607)49:3<161:AID-JMV2>3.0.CO;2-2
- Jafri, H. S., Wu, X., Makari, D., and Henrickson, K. J. (2013). Distribution of respiratory syncytial virus subtypes A and B among infants presenting to the emergency department with lower respiratory tract infection or apnea. *Pediatr. Infect. Dis. J.* 32, 335–340. doi:10.1097/INF.0b013e318282603a
- Jartti, T., Lehtinen, P., Vuorinen, T., and Ruuskanen, O. (2009). Bronchiolitis: age and previous wheezing episodes are linked to viral etiology and atopic characteristics. *Pediatr. Infect. Dis. J.* 28, 311–317. doi:10.1097/INF.0b013e31818ee0c1
- Johnson, S. M., McNally, B. A., Ioannidis, I., Flano, E., Teng, M. N., Oomens, A. G., et al. (2015). Respiratory syncytial virus uses CX3CR1 as a receptor on primary human airway epithelial cultures. *PLoS Pathog.* 11, e1005318. doi:10.1371/journal.ppat.1005318
- Joshi, P., Shaw, A., Kakakios, A., and Isaacs, D. (2003). Interferon-gamma levels in nasopharyngeal secretions of infants with respiratory syncytial virus and other respiratory viral infections. *Clin. Exp. Immunol.* 131, 143–147. doi:10.1046/j.1365-2249.2003.02039.x
- Kim, C. K., Callaway, Z., Koh, Y. Y., Kim, S. H., and Fujisawa, T. (2012). Airway IFN- $\gamma$  production during RSV bronchiolitis is associated with eosinophilic inflammation. *Lung* 190, 183–188. doi:10.1007/s00408-011-9349-5

- Kim, T. H., Oh, D. S., Jung, H. E., Chang, J., and Lee, H. K. (2019). Plasmacytoid dendritic cells contribute to the production of IFN- $\beta$  via TLR7-MyD88-dependent pathway and CTL priming during respiratory syncytial virus infection. *Viruses* 11, 730. doi:10.3390/v11080730
- Krampera, M., Vinante, F., Tavecchia, L., Morosato, L., Chilosi, M., Romagnani, S., et al. (1999). Progressive polarization towards a T helper/cytotoxic type-1 cytokine pattern during age-dependent maturation of the immune response inversely correlates with CD30 cell expression and serum concentration. *Clin. Exp. Immunol.* 117, 291–297. doi:10.1046/j.1365-2249.1999.00977.x
- Kristjansson, S., Bjarnarson, S. P., Wennergren, G., Palsdottir, A. H., Arnadottir, T., Haraldsson, A., et al. (2005). Respiratory syncytial virus and other respiratory viruses during the first 3 months of life promote a local TH2-like response. *J. Allergy Clin. Immunol.* 116, 805–811. doi:10.1016/j.jaci.2005.07.012
- Kumar, H., Kawai, T., and Akira, S. (2011). Pathogen recognition by the innate immune system. *Int. Rev. Immunol.* 30, 16–34. doi:10.3109/08830185.2010.529976
- Kurt-Jones, E. A., Popova, L., Kwinn, L., Haynes, L. M., Jones, L. P., Tripp, R. A., et al. (2000). Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat. Immunol.* 1, 398–401. doi:10.1038/80833
- Laham, F. R., Mansbach, J. M., Piedra, P. A., Hasegawa, K., Sullivan, A. F., Espinola, J. A., et al. (2017). Clinical profiles of respiratory syncytial virus subtypes A and B among children hospitalized with bronchiolitis. *Pediatr. Infect. Dis. J.* 36, 808–810. doi:10.1097/INF.0000000000001596
- Law, B. J., Langley, J. M., Allen, U., Paes, B., Lee, D. S., Mitchell, I., et al. (2004). The Pediatric Investigators Collaborative Network on Infections in Canada study of predictors of hospitalization for respiratory syncytial virus infection for infants born at 33 through 35 completed weeks of gestation. *Pediatr. Infect. Dis. J.* 23, 806–814. doi:10.1097/01.inf.0000137568.71589.bd
- Lay, M. K., González, P. A., León, M. A., Céspedes, P. F., Bueno, S. M., Riedel, C. A., et al. (2013). Advances in understanding respiratory syncytial virus infection in airway epithelial cells and consequential effects on the immune response. *Microbes Infect.* 15, 230–242. doi:10.1016/j.micinf.2012.11.012
- Lay, M. K., Bueno, S. M., Galvez, N., Riedel, C. A., and Kalergis, A. M. (2016). New insights on the viral and host factors contributing to the airway pathogenesis caused by the respiratory syncytial virus. *Crit. Rev. Microbiol.* 42, 800–812. doi:10.3109/1040841X.2015.1055711
- Legg, J. P., Hussain, I. R., Warner, J. A., Johnston, S. L., and Warner, J. O. (2003). Type 1 and type 2 cytokine imbalance in acute respiratory syncytial virus bronchiolitis. *Am. J. Respir. Crit. Care Med.* 168, 633–639. doi:10.1164/rccm.200210-1148OC
- Levine, S., Klaiber-Franco, R., and Paradiso, P. R. (1987). Demonstration that glycoprotein G is the attachment protein of respiratory syncytial virus. *J. Gen. Virol.* 68 (9), 2521–2524. doi:10.1099/0022-1317-68-9-2521
- Levine, D. A., Platt, S. L., Dayan, P. S., Macias, C. G., Zorc, J. J., Krief, W., et al. (2004). Risk of serious bacterial infection in young febrile infants with respiratory syncytial virus infections. *Pediatrics* 113, 1728–1734. doi:10.1542/peds.113.6.1728
- Lindell, D. M., Morris, S., Mukherjee, S., and Lukacs, N. W. (2009). TLR-7 mediated responses to respiratory syncytial virus (RSV). *J. Immunol.* 182 (1\_ Supplement), 133.45. doi:10.4049/jimmunol.182.Supp.133.45
- Liu, P., Jamaluddin, M., Li, K., Garofalo, R. P., Casola, A., and Brasier, A. R. (2007). Retinoic acid-inducible gene I mediates early antiviral response and Toll-like receptor 3 expression in respiratory syncytial virus-infected airway epithelial cells. *J. Virol.* 81, 1401–1411. doi:10.1128/JVI.01740-06
- Lukacs, N. W., Moore, M. L., Rudd, B. D., Berlin, A. A., Collins, R. D., Olson, S. J., et al. (2006). Differential immune responses and pulmonary pathophysiology are induced by two different strains of respiratory syncytial virus. *Am. J. Pathol.* 169, 977–986. doi:10.2353/ajpath.2006.051055
- Lukacs, N. W., Smit, J. J., Mukherjee, S., Morris, S. B., Nunez, G., and Lindell, D. M. (2010). Respiratory virus-induced TLR7 activation controls IL-17-associated increased mucus via IL-23 regulation. *J. Immunol.* 185, 2231–2239. doi:10.4049/jimmunol.1000733
- Marzec, J., Cho, H. Y., High, M., Mccaw, Z. R., Polack, F., and Kleeberger, S. R. (2019). Toll-like receptor 4-mediated respiratory syncytial virus disease and lung transcriptomics in differentially susceptible inbred mouse strains. *Physiol. Genomics* 51, 630–643. doi:10.1152/physiolgenomics.00101.2019
- Mazur, N. I., Higgins, D., Nunes, M. C., Melero, J. A., Langedijk, A. C., Horsley, N., et al. (2018). The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates. *Lancet Infect. Dis.* 18, e295–e311. doi:10.1016/S1473-3099(18)30292-5
- Mazur, N. I., Terstappen, J., Baral, R., Bardaji, A., Beutels, P., Buchholz, U. J., et al. (2023). Respiratory syncytial virus prevention within reach: the vaccine and monoclonal antibody landscape. *Lancet Infect. Dis.* 23, e2–e21. doi:10.1016/S1473-3099(22)00291-2
- Mcintosh, E. D., De Silva, L. M., and Oates, R. K. (1993). Clinical severity of respiratory syncytial virus group A and B infection in Sydney, Australia. *Pediatr. Infect. Dis. J.* 12, 815–819. doi:10.1097/00006454-199310000-00004
- Meng, J., Stobart, C. C., Hotard, A. L., and Moore, M. L. (2014). An overview of respiratory syncytial virus. *PLoS Pathog.* 10, e1004016. doi:10.1371/journal.ppat.1004016
- Miller, A. L., Bowlin, T. L., and Lukacs, N. W. (2004). Respiratory syncytial virus-induced chemokine production: linking viral replication to chemokine production *in vitro* and *in vivo*. *J. Infect. Dis.* 189, 1419–1430. doi:10.1086/382958
- Mobbs, K. J., Smyth, R. L., O'hea, U., Ashby, D., Ritson, P., and Hart, C. A. (2002). Cytokines in severe respiratory syncytial virus bronchiolitis. *Pediatr. Pulmonol.* 33, 449–452. doi:10.1002/ppul.10101
- Mochizuki, H., Todokoro, M., and Arakawa, H. (2009). RSV virus-induced inflammation and the intracellular glutathione redox state in cultured human airway epithelial cells. *Inflammation* 32, 252–264. doi:10.1007/s10753-009-9128-0
- Motegi, A., Kinoshita, M., Sato, K., Shinomiya, N., Ono, S., Nonoyama, S., et al. (2006). An *in vitro* Shwartzman reaction-like response is augmented age-dependently in human peripheral blood mononuclear cells. *J. Leukoc. Biol.* 79, 463–472. doi:10.1189/jlb.0705396
- Nielsen, H. E., Siersma, V., Andersen, S., Gahrn-Hansen, B., Mordhorst, C. H., Nørgaard-Pedersen, B., et al. (2003). Respiratory syncytial virus infection-risk factors for hospital admission: a case-control study. *Acta Paediatr.* 92, 1314–1321. doi:10.1111/j.1651-2227.2003.tb00502.x
- Ochola, R., Sande, C., Fegan, G., Scott, P. D., Medley, G. F., Cane, P. A., et al. (2009). The level and duration of RSV-specific maternal IgG in infants in Kilifi Kenya. *PLoS One* 4, e8088. doi:10.1371/journal.pone.0008088
- O'donnell, D. R., Mcgarvey, M. J., Tully, J. M., Balfour-Lynn, I. M., and Openshaw, P. J. (1998). Respiratory syncytial virus RNA in cells from the peripheral blood during acute infection. *J. Pediatr.* 133, 272–274. doi:10.1016/s0022-3476(98)70234-3
- Okabayashi, T., Kojima, T., Masaki, T., Yokota, S., Imaizumi, T., Tsutsumi, H., et al. (2011). Type-III interferon, not type-I, is the predominant interferon induced by respiratory viruses in nasal epithelial cells. *Virus Res.* 160, 360–366. doi:10.1016/j.virusres.2011.07.011
- Olshewska-Pazdrak, B., Casola, A., Saito, T., Alam, R., Crowe, S. E., Mei, F., et al. (1998). Cell-specific expression of RANTES, MCP-1, and MIP-1 $\alpha$  by lower airway epithelial cells and eosinophils infected with respiratory syncytial virus. *J. Virol.* 72, 4756–4764. doi:10.1128/JVI.72.6.4756-4764.1998
- Palomo, C., Cane, P. A., and Melero, J. A. (2000). Evaluation of the antibody specificities of human convalescent-phase sera against the attachment (G) protein of human respiratory syncytial virus: influence of strain variation and carbohydrate side chains. *J. Med. Virol.* 60, 468–474. doi:10.1002/(sici)1096-9071(200004)60:4<468:aid-jmvi16>3.0.co;2-e
- Pangesti, K. N. A., Abd El Ghany, M., Walsh, M. G., Kesson, A. M., and Hill-Cawthorne, G. A. (2018). Molecular epidemiology of respiratory syncytial virus. *Rev. Med. Virology* 28, e1968. doi:10.1002/rmv.1968
- Papi, A., Ison, M. G., Langley, J. M., Lee, D. G., Leroux-Roels, I., Martinon-Torres, F., et al. (2023). Respiratory syncytial virus prefusion F protein vaccine in older adults. *N. Engl. J. Med.* 388, 595–608. doi:10.1056/NEJMoa2209604
- Park, H. W., Lee, B. S., Kim, A. R., Yoon, H. S., Kim, B. I., Song, E. S., et al. (2012). Epidemiology of respiratory syncytial virus infection in infants born at less than thirty-five weeks of gestational age. *Pediatr. Infect. Dis. J.* 31, e99–e104. doi:10.1097/INF.0b013e318257f619
- Parrott, R. H., Kim, H. W., Arrobbio, J. O., Hodes, D. S., Murphy, B. R., Brandt, C. D., et al. (1973). Epidemiology of respiratory syncytial virus infection in Washington, D.C. II. Infection and disease with respect to age, immunologic status, race and sex. *Am. J. Epidemiol.* 98, 289–300. doi:10.1093/oxfordjournals.aje.a121558
- Peret, T. C., Hall, C. B., Schnabel, K. C., Golub, J. A., and Anderson, L. J. (1998). Circulation patterns of genetically distinct group A and B strains of human respiratory syncytial virus in a community. *J. Gen. Virol.* 79 (9), 2221–2229. doi:10.1099/0022-1317-79-9-2221
- Piedimonte, G., and Perez, M. K. (2014). Respiratory syncytial virus infection and bronchiolitis. *Pediatr. Rev.* 35, 519–530. doi:10.1542/pir.35-12-519
- Piedra, P. A., Jewell, A. M., Cron, S. G., Atmar, R. L., and Glezen, W. P. (2003). Correlates of immunity to respiratory syncytial virus (RSV) associated-hospitalization: establishment of minimum protective threshold levels of serum neutralizing antibodies. *Vaccine* 21, 3479–3482. doi:10.1016/s0264-410x(03)00355-4
- Pinto, R. A., Arredondo, S. M., Bono, M. R., Gaggero, A. A., and Diaz, P. V. (2006). T helper 1/T helper 2 cytokine imbalance in respiratory syncytial virus infection is associated with increased endogenous plasma cortisol. *Pediatrics* 117, e878–e886. doi:10.1542/peds.2005-2119
- Qiu, X., Xu, S., Lu, Y., Luo, Z., Yan, Y., Wang, C., et al. (2022). Development of mRNA vaccines against respiratory syncytial virus (RSV). *Cytokine Growth Factor Rev.* 68, 37–53. doi:10.1016/j.cytogfr.2022.10.001

- Rayavara, K., Kurosky, A., and Hosakote, Y. M. (2022). Respiratory syncytial virus infection induces the release of transglutaminase 2 from human airway epithelial cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* 322, L1–L12. doi:10.1152/ajplung.00013.2021
- Resch, B., Gusenleitner, W., and Mueller, W. D. (2007). Risk of concurrent bacterial infection in preterm infants hospitalized due to respiratory syncytial virus infection. *Acta Paediatr.* 96, 495–498. doi:10.1111/j.1651-2227.2007.00226.x
- Roca, A., Abacassamo, F., Loscertales, M. P., Quintó, L., Gómez-Olivé, X., Fenwick, F., et al. (2002). Prevalence of respiratory syncytial virus IgG antibodies in infants living in a rural area of Mozambique. *J. Med. Virol.* 67, 616–623. doi:10.1002/jmv.10148
- Rudd, B. D., Burstein, E., Duckett, C. S., Li, X., and Lukacs, N. W. (2005). Differential role for TLR3 in respiratory syncytial virus-induced chemokine expression. *J. Virol.* 79, 3350–3357. doi:10.1128/JVI.79.6.3350-3357.2005
- Rudd, B. D., Smit, J. J., Flavell, R. A., Alexopoulou, L., Schaller, M. A., Gruber, A., et al. (2006). Deletion of TLR3 alters the pulmonary immune environment and mucus production during respiratory syncytial virus infection. *J. Immunol.* 176, 1937–1942. doi:10.4049/jimmunol.176.3.1937
- Russell, C. D., Unger, S. A., Walton, M., and Schwarze, J. (2017). The human immune response to respiratory syncytial virus infection. *Clin. Microbiol. Rev.* 30, 481–502. doi:10.1128/CMR.00090-16
- Sawada, A., and Nakayama, T. (2016). Experimental animal model for analyzing immunobiological responses following vaccination with formalin-inactivated respiratory syncytial virus. *Microbiol. Immunol.* 60, 234–242. doi:10.1111/1348-0421.12365
- Schmidt, M. E., Knudson, C. J., Hartwig, S. M., Pewe, L. L., Meyerholz, D. K., Langlois, R. A., et al. (2018). Memory CD8 T cells mediate severe immunopathology following respiratory syncytial virus infection. *PLoS Pathog.* 14, e1006810. doi:10.1371/journal.ppat.1006810
- Segovia, J., Sabbah, A., Mgbemena, V., Tsai, S. Y., Chang, T. H., Berton, M. T., et al. (2012). TLR2/MyD88/NF- $\kappa$ B pathway, reactive oxygen species, potassium efflux activates NLRP3/ASC inflammasome during respiratory syncytial virus infection. *PLoS One* 7, e29695. doi:10.1371/journal.pone.0029695
- Semple, M. G., Dankert, H. M., Ebrahimi, B., Correia, J. B., Booth, J. A., Stewart, J. P., et al. (2007). Severe respiratory syncytial virus bronchiolitis in infants is associated with reduced airway interferon gamma and substance P. *PLoS One* 2, e1038. doi:10.1371/journal.pone.0001038
- Sheeran, P., Jafri, H., Carubelli, C., Saavedra, J., Johnson, C., Krisher, K., et al. (1999). Elevated cytokine concentrations in the nasopharyngeal and tracheal secretions of children with respiratory syncytial virus disease. *Pediatr. Infect. Dis. J.* 18, 115–122. doi:10.1097/00006454-199902000-00007
- Shen, Z., Zhang, Y., Li, H., and Du, L. (2022). Rapid typing diagnosis and clinical analysis of subtypes A and B of human respiratory syncytial virus in children. *Virol. J.* 19, 15. doi:10.1186/s12985-022-01744-y
- Shi, T., McAllister, D. A., O'Brien, K. L., Simoes, E. A. F., Madhi, S. A., Gessner, B. D., et al. (2017). Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet* 390, 946–958. doi:10.1016/S0140-6736(17)30938-8
- Shingai, M., Azuma, M., Ebihara, T., Sasai, M., Funami, K., Ayata, M., et al. (2008). Soluble G protein of respiratory syncytial virus inhibits Toll-like receptor 3/4-mediated IFN- $\beta$  induction. *Int. Immunol.* 20, 1169–1180. doi:10.1093/intimm/dxn074
- Smit, J. J., Rudd, B. D., and Lukacs, N. W. (2006). Plasmacytoid dendritic cells inhibit pulmonary immunopathology and promote clearance of respiratory syncytial virus. *J. Exp. Med.* 203, 1153–1159. doi:10.1084/jem.20052359
- Sourimant, J., Rameix-Welti, M. A., Gaillard, A. L., Chevret, D., Galloux, M., Gault, E., et al. (2015). Fine mapping and characterization of the L-polymerase-binding domain of the respiratory syncytial virus phosphoprotein. *J. Virol.* 89, 4421–4433. doi:10.1128/JVI.03619-14
- Stier, M. T., Bloodworth, M. H., Toki, S., Newcomb, D. C., Goleniewska, K., Boyd, K. L., et al. (2016). Respiratory syncytial virus infection activates IL-13-producing group 2 innate lymphoid cells through thymic stromal lymphopoietin. *J. Allergy Clin. Immunol.* 138, 814–824.e11. doi:10.1016/j.jaci.2016.01.050
- Stockman, L. J., Curns, A. T., Anderson, L. J., and Fischer-Langley, G. (2012). Respiratory syncytial virus-associated hospitalizations among infants and young children in the United States, 1997–2006. *Pediatr. Infect. Dis. J.* 31, 5–9. doi:10.1097/INF.0b013e31822e68e6
- Sun, Z., Pan, Y., Jiang, S., and Lu, L. (2013). Respiratory syncytial virus entry inhibitors targeting the F protein. *Viruses* 5, 211–225. doi:10.3390/v5010211
- Tabarani, C. M., Bonville, C. A., Suryadevara, M., Branigan, P., Wang, D., Huang, D., et al. (2013). Novel inflammatory markers, clinical risk factors and virus type associated with severe respiratory syncytial virus infection. *Pediatr. Infect. Dis. J.* 32, e437–e442. doi:10.1097/INF.0b013e3182a14407
- Taylor, G. (2017). Animal models of respiratory syncytial virus infection. *Vaccine* 35, 469–480. doi:10.1016/j.vaccine.2016.11.054
- Tayyari, F., Marchant, D., Moraes, T. J., Duan, W., Mastrangelo, P., and Hegele, R. G. (2011). Identification of nucleolin as a cellular receptor for human respiratory syncytial virus. *Nat. Med.* 17, 1132–1135. doi:10.1038/nm.2444
- Teng, M. N., Whitehead, S. S., Bermingham, A., St Claire, M., Elkins, W. R., Murphy, B. R., et al. (2000). Recombinant respiratory syncytial virus that does not express the NS1 or M2-2 protein is highly attenuated and immunogenic in chimpanzees. *J. Virol.* 74, 9317–9321. doi:10.1128/JVI.74.19.9317-9321.2000
- Tripp, R. A., Moore, D., Barskey, A., Jones, L., Moscattello, C., Keyserling, H., et al. (2002). Peripheral blood mononuclear cells from infants hospitalized because of respiratory syncytial virus infection express T helper-1 and T helper-2 cytokines and CC chemokine messenger RNA. *J. Infect. Dis.* 185, 1388–1394. doi:10.1086/340505
- U.S. Food and Drug Administration (2023). *FDA approves first respiratory syncytial virus (RSV) vaccine*. Available at: <https://www.fda.gov/news-events/press-announcements/fda-approves-first-respiratory-syncytial-virus-rsv-vaccine> (Accessed May 30, 2023).
- Van Bente, I. J., Van Druenen, C. M., Koopman, L. P., Kleinjan, A., Van Middelkoop, B. C., De Waal, L., et al. (2003). RSV-induced bronchiolitis but not upper respiratory tract infection is accompanied by an increased nasal IL-18 response. *J. Med. Virol.* 71, 290–297. doi:10.1002/jmv.10482
- van Druenen Littel-van den Hurk, S., and Watkiss, E. R. (2012). Pathogenesis of respiratory syncytial virus. *Curr. Opin. Virol.* 2, 300–305. doi:10.1016/j.coviro.2012.01.008
- Van Schaik, S. M., Tristram, D. A., Nagpal, I. S., Hintz, K. M., and Welliver, R. C. (1999). Increased production of IFN- $\gamma$  and cysteinyl leukotrienes in virus-induced wheezing. *J. Allergy Clin. Immunol.* 103, 630–636. doi:10.1016/s0091-6749(99)70235-6
- Vieira, R. A., Diniz, E. M., and Ceccon, M. E. (2010). Correlação entre mediadores inflamatórios na secreção nasofaríngea e no soro de crianças com infecção do trato respiratório inferior por vírus sincicial respiratório e a gravidade da doença. *J. Bras. Pneumol.* 36, 59–66. doi:10.1590/s1806-37132010000100011
- Villeneuve, R., O'donoghue, D., Thavagnanam, S., Touzelet, O., Skibinski, G., Heaney, L. G., et al. (2011). Differential cytopathogenesis of respiratory syncytial virus prototypic and clinical isolates in primary pediatric bronchial epithelial cells. *Virol. J.* 8, 43. doi:10.1186/1743-422X-8-43
- Villeneuve, R., Thavagnanam, S., Sarlang, S., Parker, J., Douglas, I., Skibinski, G., et al. (2012). *In vitro* modeling of respiratory syncytial virus infection of pediatric bronchial epithelium, the primary target of infection *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 109, 5040–5045. doi:10.1073/pnas.1110203109
- Walsh, E. E., McConnochie, K. M., Long, C. E., and Hall, C. B. (1997). Severity of respiratory syncytial virus infection is related to virus strain. *J. Infect. Dis.* 175, 814–820. doi:10.1086/513976
- Walsh, E. E., Perez Marc, G., Zareba, A. M., Falsey, A. R., Jiang, Q., Patton, M., et al. (2023). Efficacy and safety of a bivalent RSV prefusion F vaccine in older adults. *N. Engl. J. Med.* 388, 1465–1477. doi:10.1056/NEJMoa2213836
- Weigl, J. A., Puppe, W., and Schmitt, H. J. (2001). Incidence of respiratory syncytial virus-positive hospitalizations in Germany. *Eur. J. Clin. Microbiol. Infect. Dis.* 20, 0452–0459. doi:10.1007/s100960100527
- Yamaguchi, M., Sano, Y., Dapat, I. C., Saito, R., Suzuki, Y., Kumaki, A., et al. (2011). High frequency of repeated infections due to emerging genotypes of human respiratory syncytial viruses among children during eight successive epidemic seasons in Japan. *J. Clin. Microbiol.* 49, 1034–1040. doi:10.1128/JCM.02132-10
- Yerkovich, S. T., Wikström, M. E., Suriyaarachchi, D., Prescott, S. L., Upham, J. W., and Holt, P. G. (2007). Postnatal development of monocyte cytokine responses to bacterial lipopolysaccharide. *Pediatr. Res.* 62, 547–552. doi:10.1203/PDR.0b013e3181568105
- Zeng, R., Cui, Y., Hai, Y., and Liu, Y. (2012). Pattern recognition receptors for respiratory syncytial virus infection and design of vaccines. *Virus Res.* 167, 138–145. doi:10.1016/j.virusres.2012.06.003
- Zhivaki, D., Lemoine, S., Lim, A., Morva, A., Vidalain, P. O., Schandene, L., et al. (2017). Respiratory syncytial virus infects regulatory B cells in human neonates via chemokine receptor CX3CR1 and promotes lung disease severity. *Immunity* 46, 301–314. doi:10.1016/j.immuni.2017.01.010
- Zhou, H., Thompson, W. W., Viboud, C. G., Ringholz, C. M., Cheng, P. Y., Steiner, C., et al. (2012). Hospitalizations associated with influenza and respiratory syncytial virus in the United States, 1993–2008. *Clin. Infect. Dis.* 54, 1427–1436. doi:10.1093/cid/cis211