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The innate immune response to RSV: Advances in our understanding of critical viral and host factors

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Abstract

Respiratory syncytial virus (RSV) causes mild to severe respiratory illness in humans and is a major cause of hospitalizations of infants and the elderly. Both the innate and the adaptive immune responses contribute to the control of RSV infection, but despite successful viral clearance, protective immunity against RSV re-infection is usually suboptimal and infections recur. Poor understanding of the mechanisms limiting the induction of long-lasting immunity has delayed the development of an effective vaccine. The innate immune response plays a critical role in driving the development of adaptive immunity and is thus a crucial determinant of the infection outcome. Advances in recent years have improved our understanding of cellular and viral factors that influence the onset and quality of the innate immune response to RSV. These advances include the identification of a complex system of cellular sensors that mediate RSV detection and stimulate transcriptome changes that lead to virus control, and the discovery that cell stress and apoptosis participate in the control of RSV infection. In addition, it was recently demonstrated that defective viral genomes (DVGs) generated during RSV replication are the primary inducers of the innate immune. Newly discovered host pathways involved in the innate response to RSV, together with the potential generation of DVG-derived oligonucleotides, present various novel opportunities for the design of vaccine adjuvants able to induce a protective response against RSV and similar viruses.

Introduction

Respiratory syncytial virus (RSV) infections are a leading cause of respiratory illness and bronchiolitis in infants, the elderly, and the immunosuppressed[1–3]. They are a major cause of hospitalizations of asthmatics and an important cause of lung and bone marrow transplant failure[4, 5]. According to the Centers for Disease Control and Prevention, RSV is responsible for >50,000 hospitalizations and 2.1 million outpatient visits among children younger than 5 years of age, and for about 180,000 hospitalizations and 14,000 deaths among adults older than 65 years in the US alone. RSV infection induces a wide range of clinical outcomes, from a mild cold to severe respiratory illness and death, and infections of

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infants have been associated with the development of asthma, wheezing, and other chronic lung diseases later in life[6, 7]. The high degree of severe morbidity associated with RSV infections, together with its high prevalence –all humans have been infected at least once by the age of three– make this virus one of critical public health concern.

RSV is an enveloped negative-sense single-stranded RNA virus of the family *Paramyxoviridae*. It was first isolated in 1956 from a primate colony and soon after from children with bronchiolitis[8]. There are two major strains of the virus, RSV A and B, that co-circulate seasonally in the human population. RSV infections occur via inhalation of aerosolized particles or through hand to nasal transmission. The virus replicates in the epithelium of the upper respiratory tract and can spread to the lower respiratory tract, and possibly to other tissues. The RSV envelope binds and fuses with the epithelial cell membrane allowing the viral ribonucleoprotein that contains the virus genome to enter the cytosol. Viral genes are transcribed by the viral polymerase prior to genome replication in the cytosol. New infectious virions bud from the cell membrane (reviewed in [9]).

Multiple elements of the innate immune response contribute to the control of RSV infection, including diverse cell types (epithelial cells, dendritic cells, macrophages, monocytes, and granulocytes), various pattern recognition receptors (Toll like receptors (TLRs), RIG-I-like receptors (RLRs), and NOD-like receptors (NLRs)), a large array of cytokines and chemokines, cellular stress, and apoptosis. Similarly, various elements of the adaptive immune response contribute to RSV clearance including $CD4^+$ and $CD8^+$ T cells, and antibodies. Interestingly, despite successful viral clearance, protective immunity against RSV is short lived and it is common to become re-infected throughout life[10, 11]. This poor induction of long-lasting immunity has made the development of an effective vaccine a difficult task.

Intrinsic and induced innate immune mechanisms are the first line of defense against the virus. The quantity and quality of these processes are critical determinants of the infection outcome. Slow, weak, or inappropriate innate immune responses will delay virus clearance, allow the virus to replicate and spread to the lower respiratory tract, and promote the development of enhanced pathology. Inappropriate innate response will also directly impact on the quality and robustness of the adaptive immune response. Understanding of the innate immune mechanisms involved in initiating effective adaptive immune responses is also critical to identify pathways to be harnessed for the development of appropriate adjuvants and vaccines. Research in recent years has revealed new viral and host factors that influence the onset and quality of the innate immune response. These advances include new understanding of the role of host proteins and intracellular compartments in RSV sensing, the characterization of defective viral genomes (DVGs) generated during virus replication as the primary triggers of the innate immune response to RSV[12, 13], and the identification of a critical role for normal cell physiology processes, including cell stress, metabolism, and death, in mediating RSV control and clearance. This review will summarize our current understanding of these cell intrinsic and induced innate immune processes mediating the antiviral response to RSV.

Innate and cell intrinsic immune responses to RSV

RSV is recognized as foreign and potentially dangerous to the infected host by various cell intrinsic pattern recognition receptors (PRRs) that detect molecules absent in normal uninfected cells [14–16]. RSV can be detected by three major classes of PRRs: TLRs, RLRs, and NLRs. Other cellular proteins, including the protein kinase R (PKR) can also participate in RSV detection within an infected cell[16]. The quality and components of the subsequent innate immune response that will orchestrate the clearance of the virus depend on the viral product recognized and the cellular sensors involved. In general, this early immune response leads to the production of type I and III interferons (IFNs), IFN stimulated genes (ISGs), and pro-inflammatory mediators, in addition to the formation and activation of the inflammasome, cell stress, and in some cases cell death. These processes work synergistically to control the virus and to direct the development of adaptive immune responses (Figure 1).

a. PRR sensing of RSV

A major pathway for sensing RNA viruses in the cytoplasm involves the ubiquitously expressed RLRs retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) (Figure 2). While RIG-I recognizes short 5′ tri- or diphosphorylated double stranded (ds) RNA associated with specific secondary structures, MDA5 generally prefers long dsRNAs independent of their phosphorylation status (recently reviewed in[17]). Although a critical role for RLRs in initiating the innate immune response to RSV has been established using mice deficient in the RLR adaptor protein MAVS[18, 19], the specific contribution of RIG-I and MDA5 to RSV sensing is unclear and likely involves a concerted and complex series of events. While expression of both molecules is increased in nasal pharyngeal washes from infants with RSV-associated bronchiolitis, only the amount of RIG-I mRNA positively correlates with viral load[20]. In addition, human airway epithelial cell lines deficient in RIG-I or MAVS show drastically reduced activation of the type I IFN pathway in response to RSV infection[21–23]. Despite initial studies in mouse fibroblasts showing that MDA5 was irrelevant for sensing paramyxoviruses, including RSV[24], data *in vivo* and in human cells deficient in MDA5 suggest that MDA5 can sense paramyxoviruses[25–30]. A role for MDA5 in RSV sensing is suggested by intracellular localization studies showing that MDA5 is immediately recruited to cytoplasmic inclusion bodies (IBs) upon infection, limiting its ability to signal for the expression of type I IFNs[31]. Moreover, MDA5 is essential to prevent the early degradation and sustain the activation of the critical transcription factor IRF3 in human airway epithelial cells infected with RSV[30].

Unlike cytoplasmic RLRs, Toll-like receptors (TLRs) 2, 3, 4, and 7 localize in the cell membrane or within endosomal compartments and are predominantly expressed in cells of hematopoietic origin (Figure 2). Contribution of TLRs to the innate immune response to RSV has been suggested by both *in vivo* and *in vitro* experiments; however, the biological relevance of each of these molecules during natural infections is still unclear. TLR2 recognizes a variety of bacterial and viral products, and in response to RSV infection TLR2-deficient mice show reduced production of pro-inflammatory cytokines, impaired neutrophil

migration into the lung, and uncontrolled virus replication despite normal type I IFN production[32]. TLR3 and TLR7 recognize viral RNA and their deficiency skews the inflammatory environment in the lung upon RSV infection leading to enhanced mucus production and worsened lung epithelial cell hyperplasia, but does not affect viral clearance[33, 34]. TLR4 is the main sensor for bacterial lipopolysaccharides and recognizes a number of additional cellular and viral non-nucleotidic products. TLR4-deficient mice were initially shown to have delayed RSV clearance[35] and two TLR4 polymorphisms (Asp299Gly and Thr399Ile) were associated with symptomatic RSV disease in infants[36, 37]. However, a number of studies have shown no role for TLR4 in the recognition and cellular response to RSV[38–41] questioning the impact of this molecule during natural RSV infections.

Another family of PRRs encompasses the NOD-like receptors (NLRs). This family of more than 20 cytoplasmic proteins in humans is involved in various cellular processes required for the immune response to pathogens (reviewed in[42]). Some NLRs, including NLRP3, are critical for the formation of the inflammasome, a multimeric protein involved in inflammation and apoptosis through the activation of host caspases (recently reviewed in [43]). NLRP3 is activated by a number of microbial and environmental products, but no direct ligand has been described. One study showed that during RSV infection TLR2 signaling serves as the first signal for the transcription of NLRP3 genes that after translation are activated by reactive oxygen species (ROS) to form the NLRP3/ASC inflammasome[44]. Another study showed that the small hydrophobic (SH) RSV viroporin triggers NLRP3 activation by disrupting the cell membrane architecture[45]. More direct evidence for a role of NLRs in RSV detection comes from the demonstration that the NLR NOD2 binds RSV ssRNA genome and triggers the innate response after interacting with MAVS[46]. A role for NOD2 in the innate immune response to RSV is supported by a study where NOD2 deficient mice failed to produce adequate type I IFN in response to RSV infection and thus showed delayed viral clearance and enhanced lung pathology[46].

b. Type I and III IFNs

Viral recognition by PRRs leads to the downstream activation of key transcription factors including nuclear factor (NF)-kB and interferon regulatory factors (IRFs), which regulate the expression of pro-inflammatory cytokines and type I/III IFNs (for general reviews see [17, 47, 48]) (Figure 2). Type I and III IFNs are hallmark antiviral cytokines that act on their receptors in a paracrine or autocrine manner (reviewed in [49–51]) to activate a JAK/STAT-mediated signaling cascade that leads to the expression of hundreds of ISGs. These ISGs directly or indirectly interfere with viral replication and contribute to viral clearance. In mice, type I IFNs expressed in response to RSV regulate the inflammatory and antiviral response [52, 53] and mediate the recruitment of monocytes to the site of infection[54]. In addition, type I IFNs modulate the quality of the adaptive immune response against RSV by promoting the development of a Th1 type of response[55, 56]. More recently type III IFNs have been proposed to have a crucial role in virus control in the respiratory tract, as they are expressed at higher levels than type I IFNs in the nasal epithelium in mice[57] and in human airway epithelial cells[12, 58, 59]. Despite the large overlap in the genes induced by type I and III IFNs[60], further systematic research on their expression and function in the different

compartments of the respiratory tract is needed to better elucidate their contribution to the immune response to RSV.

c. IFN antagonism by RSV

The host antiviral response imposes significant evolutionary pressure on the virus. In response, viruses have evolved a number of strategies to evade immune responses. For example, paramyxoviruses tightly encapsidate their genomes into viral nucleoproteins, thereby minimizing the exposure of molecular motifs that can be recognized as foreign by PRRs. In addition, viruses produce proteins that interfere with host sensing and signaling mechanisms. RSV encodes two proteins, NS1 and NS2 that hinder the synthesis and signaling of type I and III IFNs (reviewed in [61]) (Figure 2). Deletion of the viral NS genes significantly impairs viral replication in IFN-competent cells but this loss of growth is restored in IFN-deficient cells indicating that while they are not required for virus replication, the NS proteins interfere with the antiviral IFN pathways[62–64]. NS1 and NS2 work either individually or synergistically to block almost every essential step of the IFN pathways. They promote the degradation of a number of elements of these pathways, including RIG-I, IRF3, TBK1, OASL, STAT2, and TRAF3 through the formation of a “NS-degradasome”, a heterogeneous large protein complex of 300–750 kD located in the mitochondria[65–67]. Although it is not completely clear what the degradasome consist of, NS1 can act as a functional E3 ligase and facilitate proteasomal degradation of target proteins, as shown for STAT2[67]. Interestingly, the degradasome activity *in vitro* is significantly enhanced by mitochondria motility and its association with MAVS[66]. In addition to the degradasome, NS1 and NS2 can suppress the antiviral response at many different levels, for example NS2 interacts with RIG-I, preventing its interaction with MAVS and inhibiting downstream signaling[68].

d. Cell stress-mediated antiviral responses to RSV

Host cell stress responses, including endoplasmic reticulum (ER) stress, stress granule (SG) formation, and oxidative stress, have been associated with the antiviral response to RSV[69]. ER stress results from the accumulation of unfolded or misfolded proteins in the ER lumen. ER stress triggers the activation of the unfolded protein response (UPR) that restores homeostasis. RSV, like many viral infections, induces ER stress and the UPR [70] presumably by overloading the ER/Golgi pathway with the viral glycoproteins F, G and SH[71]. The ER stress sensor inositol-requiring enzyme 1 (IRE1) is able to restrict RSV replication, evidenced by elevated RSV mRNAs and protein levels in IRE1-deficient mouse embryonic fibroblasts[72]. Although the mechanism mediating the antiviral activity of IRE1 is unclear, it is possible that its nuclease activity is responsible for selective viral mRNA degradation[73]. It is also possible that the IRE1 kinase activity modulates cellular pro-survival, pro-apoptotic and/or autophagic responses[74].

Cytoplasmic SGs composed of stalled mRNAs complexed with proteins are formed when translation is inhibited during cellular stress. RSV infection triggers the formation of SGs. Although thought to be a site of viral replication[75], evidence suggests that SGs have instead an antiviral role. In fact, RSV viral genomes are predominately located in IBs that accumulate viral proteins and trap MAVS, MDA5, and RIG-I, thereby minimizing their

ability to signal and benefiting viral replication[31]. An antiviral role for SGs is supported by studies in other viral infections[76] and by the multiple mechanisms used by RSV to inhibit their formation. Specifically, RSV redistributes the cellular protein O-linked N-acetylglucosamine transferase (OGT) needed for SG formation to IBs[77], impairs the phosphorylation of the SG assembly factor eIF2α[78, 79], and the RSV 5′ extragenic trailer sequence (5′Tr) interferes with SG assembly possibly through interaction with TIA-1-related proteins (TIAR)[80]. Although a direct antiviral role for SGs in response to RSV has not been demonstrated, it is intriguing to further understand their function and relationship with IBs during RSV infection.

Oxidative stress results from the accumulation of reactive oxygen species (ROS) within the cell. RSV infection elicits an oxidative stress response that associates with the stimulation of antiviral immunity (reviewed in [69, 81]). During RSV infection, ROS produced in response to the activity of the NADPH oxidase 2 (NOX2) activates the transcription factor NF-kB and promotes efficient RIG-I mediated IRF3 activation[82–84] while RSV interferes with expression of the dual oxidase 2 (DUOX2) and limits DUOX2-mediated production of H_2O_2 [85]. Interestingly, RSV also limits the activity of host antioxidant enzymes, thereby promoting the accumulation of high levels of ROS (reviewed in [69, 81]). High levels of ROS are associated with worsened lung pathology suggesting a delicate protective/pathogenic balance of the oxidative response during RSV infection.

e. Cell death as an antiviral response to RSV

Programmed cell death, or apoptosis, can be activated by either intrinsic (mitochondrial mediated) or extrinsic (death-receptor mediated) death signals. Apoptosis is a means to eliminate infected cells without causing excessive tissue damage. RSV infection of human lung epithelial A549 cells triggers caspase 12 activation and apoptosis in response to ER stress[70], and through promoting the expression of the inducible nitric oxide synthase (iNOS)[86]. RSV can also induce the expression of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and its receptor which sensitizes cells to cell extrinsic and intrinsic apoptotic pathways[87]. However, RSV utilizes a number of strategies to avoid or delay cell death. The RSV NS1, NS2, P and SH proteins have been shown to block or delay apoptotic death in infected cells[88–91]. RSV NS proteins protect infected cells from apoptosis by inducing the expression of AKT and other anti-apoptotic factors through a mechanism independent of suppression of IFN signaling[91]. RSV P protein impairs the extrinsic pathway of apoptosis and limits caspase 8 activation[88]. RSV SH limits the expression of TNFα and IL-1β *in vitro*[89, 90] and interacts with the pro-apoptotic protein B-cell receptor-associated protein 31 (BAP31) preventing caspase activation[92]. Furthermore, RSV infection interferes with the expression of micro221, which is required for expression of the pro-apoptotic nerve growth factor[93].

Defective viral genomes (DVGs) as the primary stimuli of the antiviral response

The development of innate and adaptive immune responses to RSV despite its versatile mechanisms to avoid innate immune activation reveals a gap on our understanding of the

factors driving the host response during natural infections. Recent data have revealed that DVGs that accumulate at the peak of virus replication are the primary triggers of the innate immune response to RSV.

DVGs are common byproducts of virus replication that result from significant deletions and alterations of the viral genome. DVGs are replication-defective and can only replicate in the presence of full-length or helper virus (recently reviewed in[13, 94]). RNA viruses produce different types of DVGs usually classified as deletion, copy-back, or snap-back depending on their sequence composition. In paramyxoviruses, copy-back DVGs promote potent antiviral immune responses characterized by the expression of high levels of type I IFNs, as well as cytokines and chemokines that mediate inflammation (Figure 3). Much of the current understanding of the immunostimulatory activity of copy-back DVGs has resulted from the study of the murine parainfluenza virus Sendai. Immunostimulatory DVGs (iDVGs) from Sendai virus trigger the antiviral response by stimulating strong signaling of intracellular PRRs RIG-I and MDA5 in infected cells[25, 95, 96]. This outstanding ability of stimulating the antiviral response in the presence of viral-encoded antagonists, and independent of type I IFN signaling, is driven by unique viral RNA secondary structures present in the DVGs[97].

RSV viral particles with a defective genome and interfering activity (also known as defective interfering particles, or DIPs) were first described in the early 1980s[98] in viruses passaged in cultured Hep-2 cells. Although *in vitro* studies have suggested a role for RSV DVGs in promoting virus persistence in cultured cells[99, 100], this was considered an artifact of *in vitro* virus replication. Recent studies from our laboratory have drastically changed this view demonstrating that DVGs are critical determinants of infection outcome. *In vitro* studies using virus stocks with a high or low content of DVGs (RSV-HD or LD, respectively) to infect cultured permissive cells, demonstrated that virus containing DVGs induced stronger expression of antiviral and pro-inflammatory genes than virus lacking DVGs[12]. The critical role of DVGs in inducing this host response was confirmed in infections with LD virus that were supplemented with purified viral particles containing DVGs. Similarly to what has been reported for other paramyxoviruses, induction of gene expression in response to RSV iDVGs during infections *in vitro* depended on signaling by intracellular viral sensor proteins of the RIG-I like receptor family and activation of key transcription factors, including IRF1[12].

RSV iDVGs are active *in vivo* and viruses with a high content of DVGs showed reduced virulence in infected Balb/c mice (Figure 3). In this infection model, RSV-HD did not promote weight loss or disease, while RSV-LD led to loss of body weight and enhanced lung pathology[12]. Most remarkably, our laboratory identified RSV DVGs in respiratory samples from infected children and the amount of DVGs detected strongly correlated with the intensity of antiviral gene expression, including interferon-induced protein with tetratricopeptide repeats 1 (IFIT1) and radical S-adenosyl methionine domain containing 2 (RSAD2), in the patient's respiratory lavage[12]. Few studies have investigated the presence of DVGs in humans and RSV DVGs are the first describe to have a critical impact on the onset of the innate antiviral response. Although the implications of these observations for the clinical outcome of infection are currently unknown and under investigation, these data

reveal that DVGs are critical players in the interaction of the virus with the host during natural infections.

Final remarks

Complex cell physiology processes and innate immune mechanisms work in concert for the early control of RSV infection. These processes also collectively signal for the development of a tailored and specific adaptive response that will clear the virus and partially protect from reinfection. An ideal vaccine will harness these mechanisms for the development of long-lasting and protective immunity. Recent advances have identified multiple host proteins involved in the early response to RSV revealing an apparent large degree of redundancy in the function of various host molecules and pathways. Regrettably, the use of mice and cells of different origins and genetic backgrounds, as well as the use of RSV virus stocks of different strains and/or different content of defective viral genomes, have complicated data interpretation and contributed to the still unclear understanding of the specific role of each molecule in the control of RSV. Further advances in our understanding depend on the development of appropriate tools and systems to study the initiation of the antiviral response during natural infections. As it is likely that multiple host factors contribute to the recognition of RSV in spatial and temporal-specific ways, the ideal system will have the complexity of the lung environment. Intriguingly, strong T cell responses occur in mice deficient in RLR and TLR signaling, despite severely impaired innate immune responses, highlighting the plasticity of the host response to RSV infection and the still significant gap in our understanding of the relevant processes that lead to virus control.

RSV has evolved effective mechanisms to evade the host response to infection that allows the virus to grow to high titers before the immune response can control it. Despite efficiently evading immune responses, innate immunity eventually develops leading to the clearance of RSV in most patients. Intriguingly, a positive correlation between the presence of IFNs and other cytokines and RSV infection in humans has been reported, supporting the ability of the host to mount an antiviral response despite viral evasion. New understanding of the critical role of viral defective genomes that are generated late during infection in triggering the antiviral response begins to explain this apparent paradox. These observations demand that we challenge the conventional view of how RSV initiates immunity, how it causes pathology, and even why the infection of RSV at early childhood associates with the exacerbation of asthma later in life.

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Abbreviations

ATF6	activated transcription factor 6
BAP31	B-cell receptor-associated protein 31

Bcl2	B-cell lymphoma 2
DR4/5	death receptor 4/5
DUOX2	dual oxidase 2
DVGs	defective viral genomes
IB	inclusion body
IFIT1	interferon-induced protein with tetratricopeptide repeats 1
iNOS	inducible nitric oxide synthase
IRE1	inositol-requiring enzyme 1
JAK-STAT	Janus kinase/signal transducers and activators of transcription
MDA5	melanoma differentiation-associated protein 5
MyD88	myeloid differentiation primary response gene 88
NLR	NOD-like receptor
NLRP3	nucleotide-binding domain and leucine-rich-repeat-containing family, pyrin domain –containing 3
NOD2	nucleotide-binding oligomerization domain containing 2
NOX2	NADPH oxidase 2
OGT	O-linked N-acetylglucosamine transferase
PKR	protein kinase R
PRRs	pattern recognition receptors
RIG-I	retinoic acid-inducible gene I
RLR	RIG-I-like receptor
ROS	reactive oxygen species
RSAD2	radical S-adenosyl methionine domain containing 2
RSV	respiratory syncytial virus
SG	stress granules
TBK1	TANK Binding Kinase 1
TIAR	TIA-1 related protein
TLR	toll like receptors
TRAFs	TNF receptor associated factors

TRAIL tumor necrosis factor-related apoptosis-inducing ligand

TRIF TIR-domain-containing adapter-inducing interferon-β

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Highlights

- Innate RSV control involves the coordinated action of various cellular processes.
- These processes include cell stress, death, and innate immune responses.
- RSV utilizes multiple mechanisms to evade the host responses.
- RSV DVGs are the primary triggers of innate immunity in mice and humans.
- New evidence suggests a critical role of DVGs in RSV pathogenesis.

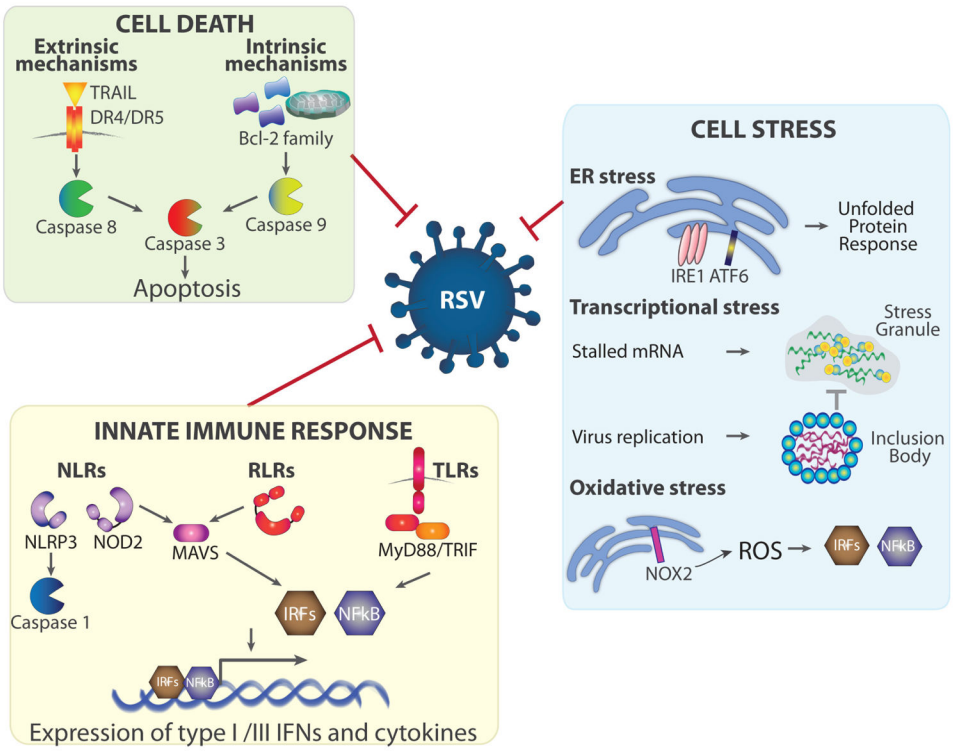

Figure 1. Innate antiviral mechanisms that control RSV replication

Respiratory syncytial virus (RSV) replication can be inhibited by various cellular responses, including cell apoptosis, innate immune responses, and cell stress responses. RSV induces both cell extrinsic and intrinsic pathways that trigger the activation of caspase 3 and cell death. These pathways are mediated by the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and its death receptor 4 and 5 (DR4/5), and by the B-cell lymphoma 2 (Bcl-2) family of proteins that localize to the mitochondria, respectively. Innate immune responses to RSV are initiated via the recognition of the virus through NOD-like receptors (NLRs), toll-like receptors (TLRs), and RIG-I-like receptors (RLRs). These molecules signal through various adaptor proteins and activate interferon regulatory factors (IRFs) and nuclear factor-kB (NF-kB) leading to the production of type I/III interferons (IFNs) and cytokines. In addition to IFN and cytokine production, the nucleotide-binding domain and leucine-rich-repeat-containing family, pyrin domain –containing 3 (NLRP3) forms the inflammasome complex and activates caspase 1 during RSV infection. RSV can induce three categories of cell stress: ER stress, transcriptional stress, and oxidative stress. Overloading the endoplasmic reticulum (ER) with RSV glycoproteins is sensed by the ER membrane protein inositol-requiring enzyme 1 (IRE1) and activated transcription factor 6 (ATF6), leading to the initiation of the unfolded protein response and control of virus replication. RSV infection triggers the formation of stress granules (SGs) containing stalled mRNAs to inhibit their translation and utilizes several mechanisms associated with inclusion bodies (IBs), where RSV replicates, to limit the formation of SGs. RSV infection leads to the production of reactive oxygen species (ROS) by the NADPH oxidase 2 (NOX2). NOX2 mediated ROS then leads to the activation of IRF3 and NF-kB and downstream antiviral responses.

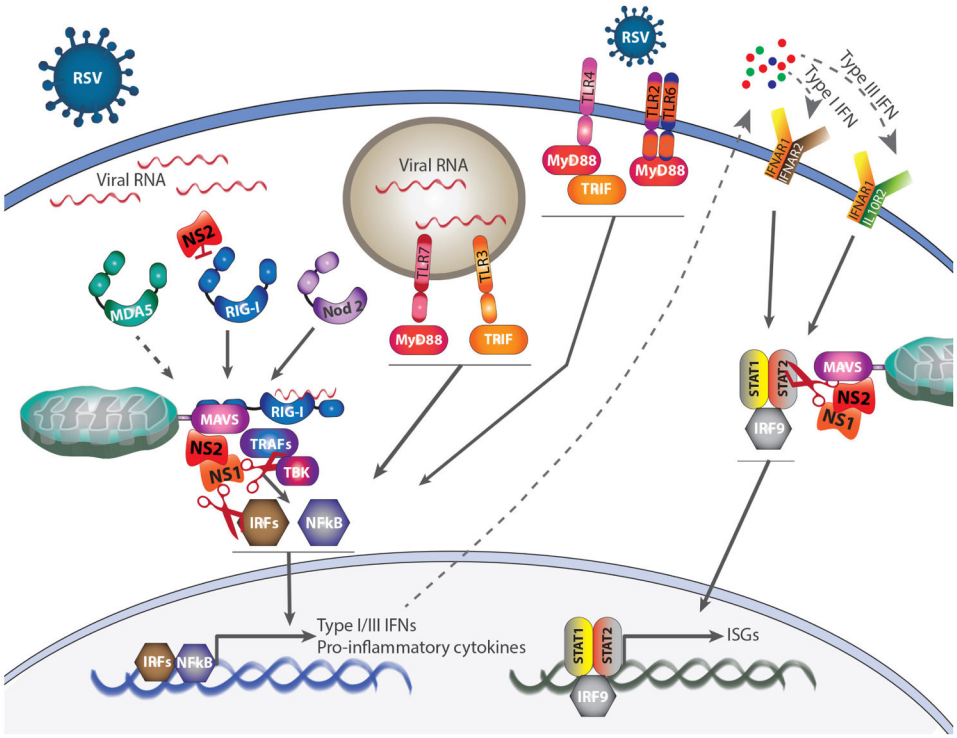

Figure 2. Mechanism of induction and inhibition of the type I and III IFN responses during RSV infection

Retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated protein 5 (MDA5), and nucleotide-binding oligomerization domain containing 2 (NOD2) are the three major cytosolic sensors that detect RSV genomes in infected cells. All three sensors utilize the mitochondria antiviral-signaling protein (MAVS) as an adaptor protein and scaffold for the transduction of downstream signaling events. Toll-like receptors (TLRs) can also sense RSV. While TLR4 and TLR2/6 heterodimers are located at the plasma membrane, TLR7 and TLR3 are located at the endosomal membrane. TLRs detect RSV ssRNA, dsRNA, or viral envelope protein and transduce the signaling via myeloid differentiation primary response gene 88 (MyD88) or TIR-domain-containing adapter-inducing interferon-β (TRIF). Pathogen recognition receptor signaling converges in the activation of interferon regulatory factors (IRFs) and nuclear factor-kB (NF-kB). The active forms of IRFs and NF-kB then translocate into the nucleus and induce the expression of type I/III IFNs and pro-inflammatory cytokines. Upon secretion, these molecules bind to their specific receptors in an autocrine or paracrine manner to stimulate the Janus kinase/signal transducers and activators of transcription (JAK-STAT) pathway and ultimately leading to the production of hundreds of interferon-stimulated genes (ISGs). RSV encodes various mechanisms to suppress these innate immune responses. Non-structural protein 1 (NS1) and NS2 are the best understood. They form the degradasome (depicted as scissors) that locates to the mitochondria and degrade multiple key proteins within the innate immune signaling pathways. Targets of the degradasome include the TNF receptor associated factors (TRAFs), TANK Binding Kinase 1 (TBK1), RIG-I, IRF3/7, and STAT2. In addition, NS2 can suppress RLR signaling via direct interaction with RIG-I.

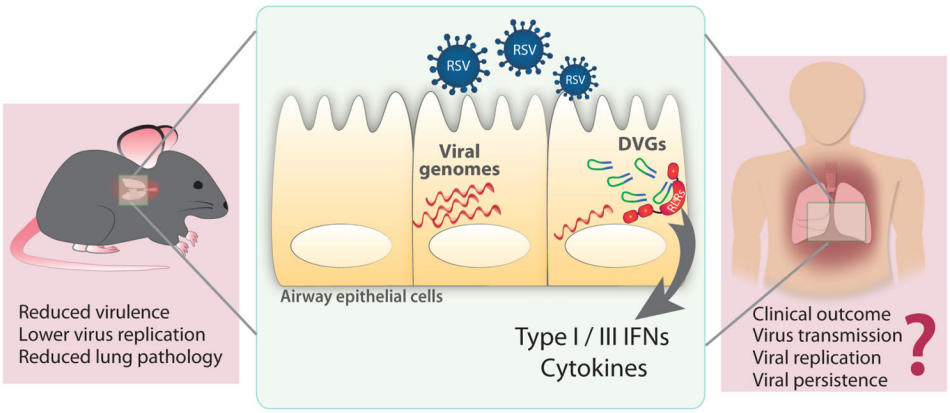

Figure 3. Impact of DVGs on RSV infection

During RSV infection, defective viral genomes (DVGs) are generated when viral genomes replicate at high levels. In cells containing DVGs, RIG-I-like receptor (RLR) signaling is activated, leading to the potent production of type I/III and pro-inflammatory cytokines. The elevated innate immune responses induced by RSV DVGs lead to reduced weight loss, lower viral replication, and mitigated lung pathology in mice. DVGs are also detected in nasal secretions from RSV infected patients. Due to their protective role in mice, it is predicted that DVGs impact the clinical outcome, viral transmission, viral replication and viral persistence in humans. These predicted functions are denoted with a question mark in the figure.