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Review

# Pattern recognition receptors for respiratory syncytial virus infection and design of vaccines

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ABSTRACT

Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract illness in infants and young children. Host immune response has been implicated in both the protection and immunopathological mechanisms. Pattern recognition receptors (PRRs) expressed on innate immune cells during RSV infection recognize the RSV-associated molecular patterns and activate innate immune cells as well as mediate airway inflammation, protective immune response, and pulmonary immunopathology. The resident and recruited innate immune cells play important roles in the protection and pathogenesis of an RSV disease by expressing these PRRs. Agonist-binding PRRs are the basis of many adjuvants that are essential for most vaccines. In the present review, we highlight recent advances in the innate immune recognition of and responses to RSV through PRRs, including toll-like receptors (TLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). We also describe the role of PRRs in the design of RSV vaccines.



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## 1. Introduction

Respiratory syncytial virus (RSV) is the most important cause of severe lower respiratory tract infection in infants and young children worldwide (Collins et al., 2001). Recent evidence suggests that RSV is an increasing cause of morbidity and mortality in the elderly and transplant patients as well as patients with chronic obstructive pulmonary disease (Thompson et al., 2003). Despite the importance of RSV as a respiratory pathogen, no licensed vaccines and effective therapy strategy are available, although palivizumab, a humanized monoclonal antibody, has been used for immunoprophylaxis against RSV in high-risk infants and young children (The IMpact-RSV Study Group, 1998). Understanding of the mechanisms that maintain the respiratory illness is limited (Collins and Melero, 2011). In-depth understanding of RSV-infection mechanisms and the host immune responses involved is essential to the development of effective vaccines. The host immune response to RSV has been implicated in both the protection and immunopathological mechanisms.

Successfully protecting the host from pathogens involves rapid activation of innate immune responses that serve as the first line of defense against invading pathogens and tailors the adaptive immune responses. Activation of innate immunity depends on the recognition of pathogen-associated molecular patterns (PAMPs) that are specific for the pathogen, but absent in the host using

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pattern recognition receptors (PRRs) expressed on sentinel cells. To date, three classes of PRRs have been identified, including toll-like receptors (TLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), and nucleotide-binding oligomerization domain (NOD)-like receptor (NLRs) (Ohto et al., 2007; Kumar et al., 2009). TLRs on the cell surface and endosomal compartments recognize a wide range of PAMPs and play a central role in initiating innate immune responses. There are currently 11 known human TLRs (Leulier and Lemaitre, 2008). RLRs, such as RIG-I and MDA5, belong to the RNA helicase family and have been identified as essential cytosolic receptors for intracellular viral RNAs, mediating the anti-viral programs via type I IFN induction (Yoneyama et al., 2005). The NLRs constitute a large cytosolic receptor family. The NLR family members NOD1, NOD2, and NALP3 recognize PAMPs in the cytosol as well as play a role in innate immunity (Akira et al., 2006). The cytosolic NLR and RLR families contribute to the immune response against pathogens in collaboration with the membrane-bound TLRs (Yoneyama and Fujita T, 2008). All these PRRs recognize various PAMPs and activate transcription factors NF-κB, mitogen-activated protein kinases (MAPKs), and/or members of the interferon regulatory factor (IRF) family, which regulate the expression of inflammatory cytokines and type I interferons (Medzhitov, 2007). RSV can be recognized by these three classes of PRRs. Airway resident leukocytes, such as dendritic cells (DCs) and macrophages as well as recruited proinflammatory granulocytes (neutrophils and eosinophils), are markedly involved in mediating airway inflammation and asthma pathophysiology by expressing these PRRs during RSV infection (Amanatidou et al., 2009; Murawski et al., 2009). Adjuvants are essential for enhancing vaccine efficacy. Agonist-binding PRRs are the basis of many adjuvants. These molecules exert their adjuvant function by interacting with TLRs, NLRs, RLRs, and signal through MyD88-dependent and MyD88-independent pathways (Higgins and Mills, 2010). In the present review, we highlight recent advances in the innate immune recognition of and responses to RSV through PRRs, which resulted in appropriate antiviral responses and/or pulmonary immunopathology. We also describe the role of PRRs in the design of RSV vaccines.

## 2. TLR-mediated innate immune recognition of RSV

TLRs are found on a wide range of cells such as macrophages, DCs, epithelial cells, eosinophils, and neutrophils. TLR1, TLR2, TLR4, TLR5, and TLR6 reside on the cell surface as well as recognize microbial surface molecules and/or products, such as LPS or RSV fusion (F) protein. TLR3, TLR7, TLR8, and TLR9 are expressed in intracellular vesicles as well as recognize viral nucleic acids. Ligand recognition by TLRs, except TLR3, leads to the activation of the MyD88-dependent pathway, whereas TLR3 and TLR4 activate the TRIF-dependent pathway (Yoneyama et al., 2005). The activation of both pathways triggers a downstream signaling pathway and activates ultimately the transcription factors NF-κB and MAPK, which contribute to express multiple inflammatory cytokines and chemokines. Endosomal TLRs can also activate IRFs and induce type I IFNs (Yoneyama et al., 2005).

A number of TLRs have been linked to RSV infection, including TLR2, TLR3, TLR4, and TLR7. TLR2 is expressed on the surface of immune cells and tissues as a heterodimer complex with TLR1, TLR6, CD36, CD14, or dectin-1 to discriminate the molecular structure of the ligands. Recent studies have indicated that TLR2 is involved in RSV recognition and subsequent innate immune activation. Shimojo et al. (2006) suggested that TLR2 is a functional receptor for RSV. Murawski et al. (2009) demonstrated that TLR2 and TLR6 signaling in leukocytes could activate innate immune response to RSV by promoting proinflammatory cytokines and chemokines production, neutrophil migration, and DCs activation as well as control viral replication in the lung.

TLR3 is an intracellular receptor that recognizes double-stranded RNA (dsRNA). TLR3-mediated response is channeled through toll-interleukin (IL)-1 receptor domain-containing adaptor inducing TRIF, activates both NF-κB and IRF-3, and subsequently drives production of IFN-β, CXCL10, CCL12, and CCL5 (Bermejo-Martin et al., 2007). TLR3 can detect the dsRNA generated during the RSV replication cycle (Aeffner et al., 2011). TLR3 expression might be necessary to regulate the immune environment that contributes to RSV-associated pulmonary immunopathology, although TLR3 has no or little effect on RSV clearance (Rudd et al., 2005; Rudd et al., 2006; Groskreutz et al., 2006). Rudd et al. (2005) showed that RSV 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 upregulates TLR3 expression in RSV-infected cells. The activation of TLR3 during RSV infection promotes a predominant Th1-type response (Rudd et al., 2006). By contrast, the deletion of TLR3 leads to increased pathogenic Th2-biased response, including IL-13 and IL-5 production, mucus overproduction, and an accumulation of eosinophils in the airways (Rudd et al., 2006). RSV infection increases TLR3 expression in the respiratory epithelial cells, which sensitizes the epithelial cells to subsequent extracellular dsRNA exposure through activation of the inflammation-related transcription factor NF-κB and production of the chemokine IL-8 (Groskreutz et al., 2006). These results are consistent with the notion that the persistence of RSV-inducing inflammatory responses may provide a substratum for later challenges with other pathogens (Resch et al., 2007). The TLR3 signaling pathway may be an attractive therapeutic target for RSV-induced lung inflammation and subsequent allergic asthma.

TLR4 is the first to be shown to have an effect on RSV infection through its interaction with RSV F protein using CD14 as a co-receptor (Kurt-Jones et al., 2000; Suzuki et al., 2008). Activation of TLR4 and CD14 by RSV F protein leads to NF-κB-mediated inflammatory and innate immune response (Haeberle et al., 2002) and increased TLR4 expression in epithelial cells (Monick et al., 2003). RSV-elicited proinflammatory cytokines IL-6 and IL-8 production by epithelial cells is suppressed through inhibition of the RSV-TLR4/CD14 interaction (Numata et al., 2010). TLR4 null mice challenged with RSV showed reduced pulmonary NK and $CD14^+$ cell trafficking, deficient NK cell function, impaired IL-12 expression and delayed clearance of RSV compared with TLR4-positive mice (Haynes et al., 2001; Kurt-Jones et al., 2000). The two single nucleotide polymorphisms (SNPs) encoding Asp299Gly and Thr399Ile substitutions in the TLR4 ectodomain have been linked epidemiologically with an increased risk of severe RSV bronchiolitis and increased risk for hospitalization in previously healthy infants (Mandelberg et al., 2006; Tal et al., 2004). Both SNPs are highly associated with symptomatic RSV disease in premature infants, a largely high-risk population, suggesting that a fully functional TLR4 response is central to the development of an efficacious innate immune response to natural RSV infection, particularly in high-risk infants (Awomoyi et al., 2007). Tulic et al. (2007) demonstrated that human bronchial epithelium expressing 299Gly or 399Ile display normal levels of intracellular TLR4 but failed to translocate efficiently the receptor to the cell surface, and therefore attenuated ligand binding, consequently impairing NF-κB signaling and reducing production of IFNs, IL-8, IL-10, IL-12p35, IL-18, CCL8, and TNF-α. Their finding provided a possible mechanism by which TLR4 polymorphisms contribute to enhance susceptibility to RSV infection in these individuals. Contrast to these reports, a clinical study has shown that upregulated TLR4 expression on blood monocytes in infants is linked closely to disease severity (Gagro et al., 2004). The significant role of TLR4 in RSV infection has been also questioned