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Immune signaling by RIG-I-like receptors

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Abstract

The RIG-I-like receptors (RLRs) RIG-I, MDA5, and LGP2 play a major role in pathogen sensing of RNA virus infection to initiate and modulate antiviral immunity. The RLRs detect viral RNA ligands or processed self RNA in the cytoplasm to triggers innate immunity and inflammation and to impart gene expression that serves to control infection. Importantly, RLRs cooperate in signaling crosstalk networks with Toll-like receptors and other factors to impart innate immunity and to modulate the adaptive immune response. RLR regulation occurs at a variety of levels ranging from autoregulation to ligand and co-factor interactions and post-translational modifications. Abberant RLR signaling or dysregulation of RLR expression is now implicated in the development of autoimmune diseases. Understanding the processes of RLR signaling and response will provide insights to guide RLR-targeted therapeutics for antiviral and immune modifying applications.

RIG-I like receptors (RLRs) are a family of DExD/H box RNA helicases that function as cytoplasmic sensors of pathogen-associated molecular patterns (PAMPs) within viral RNA (reviewed in (Onoguchi, Yoneyama et al. 2011)). The RLRs signal downstream transcription factor activation to drive type 1 interferon (IFN) production and antiviral gene expression that elicits an intracellular immune response to control virus infection. To date, three RLR members have been identified: RIG-I (retinoic acid-inducible gene I) – the founding member and therefore best characterized of this family, MDA5 (melanoma differentiation associated factor 5), and LGP2 (laboratory of genetics and physiology 2 and a homolog of mouse D11lgp2).

The three RLRs are broadly expressed in most tissues where they signal innate immune activation in a variety of cell types. While they play a prominent role in triggering innate defenses within myeloid cells, epithelial cells, and cells of the central nervous system, their actions are not essential for IFN production in plasmacytoid dendritic cells despite their expression in this cell type. RLR expression is typically maintained at low levels in resting cells but is greatly increased with IFN exposure and after virus infection (Kang, Gopalkrishnan et al. 2004; Yoneyama, Kikuchi et al. 2004; Imaizumi, Kumagai et al. 2005; Yoneyama, Kikuchi et al. 2005). Further, MDA5 expression was shown to be virus-inducible in cells lacking the IFN receptor, suggesting that RLR expression can be driven by a direct virus-inducible signal (Yount, Moran et al. 2007). The priming of cells with IFN or ectopic expression of the RLRs dramatically sensitizes them for PAMP recognition and immune signaling (Yoneyama, Kikuchi et al. 2004; Sumpter, Loo et al. 2005; Yoneyama,

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Kikuchi et al. 2005), suggesting that RLR function is in part regulated by their respective expression. Consistent with this idea, pluripotent cells noted to have attenuated interferon response to cytoplasmic RNA PAMPs express little or no RLRs, thus rendering them refractory to cytoplasmic PAMP detection and signaling (Chen, Yang et al. 2010).

RIG-I and MDA5 detect a variety of viruses and signal the production of IFN and induction of an antiviral response. They share a number of structural similarities (Figure 1) including their organization into three distinct domains: i) an N-terminal region consisting of tandem caspase activation and recruitment domains (CARD), ii) a central DExD/H box RNA helicase domain with the capacity to hydrolyze ATP and to bind and possibly unwind RNA, and iii) a C-terminal repressor domain (RD) embedded within the C-terminal domain (CTD) that in the case of RIG-I is involved in autoregulation (Yoneyama, Kikuchi et al. 2004; Yoneyama, Kikuchi et al. 2005; Saito, Hirai et al. 2007). Although similarly organized, LGP2 lacks the N-terminal CARDs and is currently thought to function as a regulator of RIG-I and MDA5 signaling (Yoneyama, Kikuchi et al. 2005).

Pathogen sensing by the RLRs

Members of the RLR family have been implicated in the recognition of a variety of viruses, and a list of these viruses is summarized in Table 1. Various studies have shown that RIG-I confers recognition of the hepaciviruses and members of the Paramyxoviridae, Rhabdoviridae, and Orthomyxoviridae virus genera, while MDA5 is associated with the detection of members of the Picornaviridae (Kato, Takeuchi et al. 2006). Thus, mice lacking either RIG-I or MDA5 become highly susceptible to RNA virus infection (Kato, Takeuchi et al. 2006; Venkataraman, Valdes et al. 2007; Satoh, Kato et al. 2010). In contrast, a subset of viruses including Dengue virus, West Nile virus and reovirus present PAMPs that are recognized during acute infection by both MDA5 and RIG-I (Fredericksen, Keller et al. 2008; Loo, Fornek et al. 2008). Moreover, MDA5 has recently been implicated in the recognition of murine norovirus (McCartney, Thackray et al. 2008). Although LGP2 possesses the ability to bind RNA, it has yet to be shown to be involved in the actual detection of viral RNA during infection (Yoneyama, Kikuchi et al. 2005).

RIG-I was initially characterized as a dsRNA-binding protein that triggered IFN induction and virus signaling in response to the synthetic dsRNA poly(I:C) (Yoneyama, Kikuchi et al. 2004), and was then identified as a major factor controlling cell permissiveness for hepatitis C virus replication (Sumpter, Loo et al. 2005). Several studies have since led to the characterization of molecular features involved in the activation of RIG-I-dependent signaling (further reviewed by (Schlee, Hartmann et al. 2009; Schlee and Hartmann 2010)). RIG-I preferentially recognizes RNA sequences marked with 5′ triphosphorylated (5′ppp) ends, which serve in part to define a non-self RNA PAMP (Hornung, Ellegast et al. 2006). Removal of the 5′ppp completely from a PAMP RNA abrogates signaling, whereas diphosphate or monophosphate modifications from 5′ppp severely attenuate signaling (Hornung, Ellegast et al. 2006; Kim, Hwang et al. 2008). Studies based on the influenza virus genomic RNA led to the conclusion that independent of length, at least one phosphate at the 5′ end of the RNA is required to trigger RIG-I-dependent signaling but the 5′ppp is required for full signaling potential by RIG-I (Pichlmair, Schulz et al. 2006). Next generation sequencing of RNA derived from RIG-I/RNA complexes isolated from influenza virus infected cells confirm that RIG-I associates preferentially with short 5′ppp-RNA sequence motifs along RNA containing some dsRNA regions (Baum, Sachidanandam et al. 2010). Comparison of RIG-I and MDA5 interaction with synthetic dsRNA poly(I:C) suggest that whereas MDA5 preferentially recognizes high molecular weight poly(I:C) fragments, RIG-I shows a preference for shorter RNA fragments and can also bind to ssRNA (Kato, Takeuchi et al. 2008). Consistent with these observations, Marques et al. reported that blunt-

end dsRNA fragments as short as 23bp can trigger RIG-I-dependent signaling, and that RIG-I has a preference for recognizing blunt ended dsRNA over those with 5′- or 3′- overhangs (Marques, Devosse et al. 2006). Moreover, as ssRNA predicted to impose limited or no secondary structure but containing 5′ppp can also serve as a potent PAMP ligand of RIG-I, it is likely that RIG-I can interact with various RNA substrates based on the presence of a 5′ppp marking the RNA as potential non-self PAMP. However, it should be noted that a synthetic 5′ppp-ssRNA failed to drive RIG-I signal activation in the absence of at least a short complementary sequence or polynucleotide motifs (see below), suggesting that 5′ppp alone could be insufficient as a determinant of non-self for RIG-I recognition, which may require additional motifs marking a RNA as non-self (Schlee, Roth et al. 2009).

RNA ligand specifications

A number of studies suggest that sequence composition of an RNA ligand may contribute to the activation of RIG-I-dependent signaling. Two in particular reported that RIG-I preferentially signals IFN expression in response to poly-uridine motifs that containing interspersed C nucleotides (known as poly-U/UC; Saito, Owen et al. 2008) as present in the genome of hepatitis C virus (HCV) that was produced to include 5′ppp (Saito, Owen et al. 2008; Uzri and Gehrke 2009). Of note is that HCV has a ssRNA genome that is noncapped and includes a 5′ppp. Deletion of the poly-U/UC motif from the HCV genome completely abrogated RIG-I-dependent signaling despite the presence of a 5′ppp within the genome RNA, indicating that 5′ppp is not sufficient to confer RIG-I signaling induction but that additional PAMP motifs are likely to work in concert with 5′ppp to mark an RNA as a RIG-I ligand. In support of this, further analyses revealed that poly-uridine-rich RNA motifs serve to enhance RLR signaling to ssRNA PAMPs representing Ebola virus, influenza virus, and other RNA viruses (Saito, Owen et al. 2008). In the second study, Gondai et al. showed that extension of in vitro transcribed short hairpin RNAs by one G abolished IFN induction by RIG-I (Gondai, Yamaguchi et al. 2008). However, as the addition of the G results in the formation of overhangs which are inhibitory to RIG-I signaling, it is difficult to differentiate whether signaling was abolished because of sequence composition or the removal of blunt ends. Overall these studies serve to indicate that PAMP RNA ligand composition, along with 5′ppp are important determinants of a non-self signature for RIG-I recognition. Thus, 5′ppp along with the secondary motifs such as poly-uridine runs as well as specific short dsRNA structures may serve together to mark a viral RNA as non-self for recognition by RIG-I. In support of this notion, RIG-I has been reported to signal IFN induction in response to 5′ppp AU-RNA polymers that are generated by RNA polymerase III (pol III) transcription using exogenous dAdT DNA as templates (Ablasser, Bauernfeind et al. 2009; Chiu, Macmillan et al. 2009). In this manner, RIG-I is predicted to be able to respond to dsDNA from intracellular pathogens (Kumar, Kawai et al. 2006; Ablasser, Bauernfeind et al. 2009; Monroe, McWhirter et al. 2009) through recognition of a non-self product of pol III transcription. Furthermore, RNA cleavage products generated by the 2′,5′-linked oligoadenylate-activated RNase L ribonuclease can trigger RIG-I- and MDA5-dependent IFN induction, and this may serve as a means to amplify the antiviral response through production of RLR substrates (Malathi, Dong et al. 2007; Malathi, Saito et al. 2010). Of note however is that such RNase L cleavage products do not contain a 5′ppp but instead contain a 3′ monophosphate in a context of a short (<200 nt) length essential for RLR signaling (Malathi, Saito et al. 2010). MDA5 further cooperates with RNase L to signal IFN induction in response to a viral mRNA from parainfluenza 5 virus (Luthra, Sun et al. 2011). Based on the nature of RNase L cleavage products, these observations suggest that RIG-I and MDA5 can signal IFN induction through their recognition of small RNA products marked with specific cleavage or processing signatures independently of 5′ppp. However, the exact nature of the PAMP motifs recognized by the RLRs in this situation is not defined.

Based on recent studies, RIG-I ligand RNA motifs that serve as a PAMP in pathogen recognition can be described chiefly as a 5′ppp RNA encoding short motifs of PAMP recognition, including dsRNA structure, ssRNA encoding polynucleotide runs of specific length, and variable end arrangements of blunt or overhang that imparts stable binding to RIG-I. Moreover, RNA marked via endonucleolytic cleavage may offer PAMP signature through combination of length and 5′ or 3′ terminal structure. Binding of 5′ppp RNA to RIG-I has been confirmed by crystal structure analysis of the RIG-I RD, also called the C-terminal domain or CTD. In this case the 5′ppp terminus of the ligand RNA is predicted to bind to RIG-I and anchor the RNA ligand through interaction with specific charged residues within a ligand binding groove of the RD or CTD (Cui, Eisenacher et al. 2008; Takahasi, Kumeta et al. 2009; Lu, Xu et al. 2010). In contrast, synthetic dsRNA poly(I:C) is predicted to differentially bind to RIG-I via a dsRNA-interaction site within the helicase domain (Cui, Eisenacher et al. 2008). Analyses of the RD or CTD from all three RLRs suggest that they form distinct RNA-binding loops that impart the basis for ligand specificity (Takahasi, Kumeta et al. 2009). While MDA5 encodes a RD-like motif analogous to the RIG-I RD, this domain, referred as the CTD, does not impose autoregulation of MDA5 signaling as it does for RIG-I (Saito, Owen et al. 2008). However, crystal structure reveals that the MDA5 CTD assumes a highly structured fold similar to that of the RIG-I and the LGP2 RD (Li, Lu et al. 2009). NMR titration with dsRNA showed that MDA5 interaction with dsRNA occurs through electrostatic associations on a positively charged surface. Morevoer, these analyses predict that the MDA5 CTD binds preferentially to dsRNA with blunt ends and not to dsRNA with 5′ or 3′ overhangs. Although little is known about the RNA ligands for LGP2, crystal structure and NMR studies predict that LGP2 is likely to interact with a varety of RNA species (Murali, Li et al. 2008; Pippig, Hellmuth et al. 2009; Takahasi, Kumeta et al. 2009).

RLR activation and autoregulation

The mechanisms of RLR signaling control are best understood in the case of RIG-I, with little known currently on how MDA5 and LGP2 activity is regulated beyond RNA binding. Structure-function analyses of RIG-I demonstrate its signaling activity is tightly regulated by autoregulation mediated by intramolecular interactions between the CARDs and the RD (Saito, Hirai et al. 2007). These observations indicate a model of RIG-I autoregulation in which in the absence of an RNA ligand RIG-I is held in a “closed” conformation where it is predicted that the CARDs are sequestered from signaling interactions through intramolecular association with the RD (Figure 2). Based on crystal structures analyses, the 5′ppp terminus of a dsRNA ligand is predicted to bind to aa 802-925 within the RD or CTD of RIG-I through electrostatic interactions (Cui, Eisenacher et al. 2008). Subsequent conformational changes are predicted to release the CARDs from RD repression. In this “open” conformation, RIG-I then becomes signaling-active wherein it likely multimerizes and the CARDs within this RIG-I complex are now able to mediate associations with its adaptor protein, IPS-1 (also known as MAVS, VISA or Cardif) to induce IFN production and the expression of host defense genes (Kawai, Takahashi et al. 2005; Meylan, Curran et al. 2005; Seth, Sun et al. 2005; Xu, Wang et al. 2005). Consistent with this model of RLR activation, RIG-I mutants lacking the RD signal constitutively whereas those lacking the CARD exhibit dominant negative activity in response to virus and dsRNA (Yoneyama, Kikuchi et al. 2004; Yoneyama, Kikuchi et al. 2005). Biochemical and structural analyses confirm that the RD or CTD of RIG-I adopt a different fold when bound to an agonist RNA as compared to RIG-I alone or RIG-I bound to an antagonist RNA (Saito, Owen et al. 2008; Ranjith-Kumar, Murali et al. 2009; Lu, Xu et al. 2010; Lu, Ranjith-Kumar et al. 2011). Thus, RIG-I signaling initiation is controlled in part through a combination of RNA ligand-binding and conformation changes that alter self-interactions, leading to signaling induction or suppression.

As noted above, MDA5 does not encode an RD that self-governs its signaling actions. In fact, unlike RIG-I, when ectopically expressed wild type MDA5 imparts constitutive signaling without the need for a RNA ligand to stimulate its activation (Saito, Hirai et al. 2007). These observations suggest that interaction of MDA5 with specific regulatory proteins might serve to mediate its signaling control. In support of this idea, studies of Lgp2-deficient mice reveal that fibroblasts and myeloid cells from these animals have major defects in innate immune signaling induced by MDA5-specific viral or p(I:C) agonists (Venkataraman, Valdes et al. 2007; Satoh, Kato et al. 2010). Moreover, these animals exhibited attenuated responses to RIG-I-specific stimuli, suggesting that LGP2 may function as a co-factor of RLR signaling. These observations however are in contrast to in vitro studies of LGP2 structure and function in which ectopic expression of LGP2 was shown to negatively regulate RLR signaling. Although biochemical studies reveal that RNA-binding and ATP hydrolysis activities are not required by LGP2 for its negative regulation of RLR signaling (Bamming and Horvath 2009; Li, Ranjith-Kumar et al. 2009), cells that express an LGP2 mutant that has lost its ATP hydrolyzing activity are similarly impaired in signaling of IFN induction in response to RNA virus infection as are cells lacking LGP2 (Satoh, Kato et al. 2010). LGP2 encodes a functional RD that when expressed alone can impart suppression of RIG-I signaling, suggesting that LGP2 control of RLR signaling may be regulated through RD interactions with RIG-I and possibly MDA5 (Saito, Hirai et al. 2007). Thus, ATPase activity, specific RD interactions, and likely RNA ligand binding may impart LGP2 activity as a positive or negative regulator of RLR signaling. Additional studies to assess the role of each, as well as the impact of dynamic LGP2 expression levels, on RLR signaling control are needed in order to assign a role for LGP2 in innate immune signaling regulation.

The RLR signaling pathway: Effector actions and regulation

As noted above, RIG-I and MDA5 signal IFN production in response to virus infection through a common adaptor IPS-1 (Kawai, Takahashi et al. 2005; Meylan, Curran et al. 2005; Seth, Sun et al. 2005; Xu, Wang et al. 2005). IPS-1 is a membrane-associated, CARD-containing protein that is essential for RLR-dependent IFN production in response to virus infection. Signaling is initiated by the detection of viral RNA PAMPs which induces RLR activation and association with IPS-1 through homotypic CARD-CARD interactions. In addition to the N-terminal CARD which shares homology with the first CARDs of RIG-I and MDA5, sequence analysis revealed a transmembrane domain on the IPS-1 C-terminus that anchors it to intracellular membranes (Seth, Sun et al. 2005; Potter, Randall et al. 2008). Reports have placed IPS-1 on the outer membranes of the mitochondria, the membranes of peroxisomes and mitochondria-associated membranes (Seth, Sun et al. 2005; Dixit, Boulant et al. 2010; Horner, Liu et al. 2011). RIG-I and MDA5 interaction with IPS-1 serves to relocate the RLRs to IPS-1-associated membranes where they and downstream signaling molecules accumulate to form an IPS-1 signalosome that drives IFN production (Hiscott, Lacoste et al. 2006; Lin, Lacoste et al. 2006; Ohman, Rintahaka et al. 2009; Dixit, Boulant et al. 2010; Horner, Liu et al. 2011). Signal transduction culminates in the activation of a transcription program leading to IFN production and the induction of the antiviral state.

Cell-intrinsic innate immunity and IFN actions

Key transcription factors involved in RLR signaling and the IPS-1 signalosome include interferon regulatory factor 3 (IRF3), IRF7 and NF-κB (Paz, Sun et al. 2006). IRF3 and IRF7 are latent transcription factors that upon signal transduction are phosphorylated by the non-canonical IκB kinases IKKε or TBK1. Phosphorylated IRF3 and IRF7 form homo- and heterodimers that accumulate in the nuclei where they bind to target sequences to drive gene transcription. In contrast, NF-κB activation requires the IKK complex mediated

phosphorylation of its inhibitory subunit IκBα that is then subjected to ubiquitin-dependent degradation by proteasomes. Activated IRF3 and/or IRF7 and NF-κB together with the transcription complex of ATF-2 and c-Jun and the transcription enhancer CBP-p300 assemble as part of an enhanceosome to direct IFNβ transcription (Panne 2008). In most cell types except for plasmacytoid dendritic cells, IRF3 is constitutively expressed whereas IRF7 expression remain low until it is induced in the presence of IFN in a positive feedback loop (Marie, Durbin et al. 1998; Sato, Hata et al. 1998). IRF3 is therefore thought to function in the immediate early enhanceosomes in most cell types while IRF7 direct later transcription programs. IRF-3 and components of the NF-κB activation program have been identified as constituents of an IPS-1 signalosome in various studies (Hiscott, Lacoste et al. 2006; Lin, Lacoste et al. 2006; Ohman, Rintahaka et al. 2009; Dixit, Boulant et al. 2010; Horner, Liu et al. 2011).

IFNβ that is produced and secreted as a result of the RLR cascade binds to the IFN receptor in an autocrine or paracrine manner to direct JAK-STAT signaling and the ISGF3-dependent expression of interferon stimulated genes (ISGs). This signaling serves to amplify the IFN response by increasing the expression of IFN-α subtypes in a positive feedback loop. Other ISGs include those encoding proteins with direct antiviral activity such as viperin (*Cig5*), the ISG56 or IFIT family of proteins, OAS and Mx-1, immune-proteasome components involved in antigen presentation, PAMP receptors including all three RLRs and members of the Toll-like receptor (TLR) family, transcription factors such as IRF-7 as well as numerous pro-inflammatory cytokines and chemokines (Loo, Fornek et al. 2008; Poeck, Bscheider et al. 2010). The end result of ISG expression is the induction of cellular conditions and immune regulation that cooperate to control infection and the establishment of an antiviral state.

Inflammatory signaling

In addition to inducing the expression and production of IFN and ISG products, virus infection and signaling through the RLRs also induces the expression of the IFN-λ, family of IL-10-related cytokines known collectively as type III interferon (IFN-λ) (Kotenko, Gallagher et al. 2003; Sheppard, Kindsvogel et al. 2003; Coccia, Severa et al. 2004; Pestka, Krause et al. 2004; Osterlund, Veckman et al. 2005; Ank, West et al. 2006) and various pro-inflammatory cytokines to control infection (Poeck, Bscheider et al. 2010). Onuguchi et al. showed that RLR signaling through RIG-I, IPS-1, TBK1 and IRF-3 are required for the induction of IFN-λ following Newcastle disease virus (a paramyxovirus) infection (Onoguchi, Yoneyama et al. 2007), and analyses of the promoter regions upstream IFN-λ genes reveal numerous cis-acting elements for IRFs and NF-κB binding (Osterlund, Veckman et al. 2005; Onoguchi, Yoneyama et al. 2007). Taken together, the data strongly suggest that type I IFN and and IFN-λ induction both occur through similar pathways, and that RLR signaling also induces the expression IFN-λ to control virus infection. In terms of the pro-inflammatory response, recent studies suggest that RLR signaling mediates this response using two pathways. The first involves IPS-1-CARD9-Bcl-10-dependent transcription of pro-inflammatory genes, many of which are NF-κB target genes (Poeck, Bscheider et al. 2010). The second involves RIG-I association with ASC protein to trigger caspase-1-dependent inflammasome activation and the processing of pro-inflammatory cytokines such as IL-1β and IL-18 into their mature forms. Thus RLR signaling may drive bifurcation beyond or independently of IPS-1 to mediate the inflammatory response that accompanies interferon production and adaptive immunity.

Signaling cross talk

RLR signaling shares a number of components in common with other cellular pathways involved in immune protection. RLR signaling to IRF3, IRF7 and NF-κB is regulated by

complex signaling transduction events that involve components previously associated with the tumor necrosis factor receptor I (TNFRI) and TLR signaling pathways. IPS-1 interacts with the TNFR-associated death domain (TRADD) protein and its recruitment is important for RLR signaling (Michallet, Meylan et al. 2008). TRADD exists in a complex with Fas-associated death domain-containing protein (FADD), and the death domain kinase RIP1. Signaling through the IPS-1 associated TRADD-FADD-RIP1 complex results in the recruitment of TANK and NEMO to the IPS-1 signalosome to facilitate IRF3 and IRF7 activation by TBK1 or IKKi, IKKα-IKKβ-dependent activation of NF-κB, and IFN production (Guo and Cheng 2007; Zhao, Yang et al. 2007; Michallet, Meylan et al. 2008). In addition, TBK1 and IKKε associate with the adaptor proteins NAP1 and SINTBAD, (Sasai, Shingai et al. 2006; Guo and Cheng 2007; Ryzhakov and Randow 2007). Although the relationship between these molecules is unclear, knockdown of NAP1 or SINTBAD impaired virus-induced signaling through both the TLR and RLR pathways. A recent discovery of novel human IKKε splice variants show that the splice variants vary in their ability to interact with TANK, NAP1, and SINTBAD, and this may serve as a basis for directing the different signaling functions of IKKε within the RLR signaling program (Koop, Lepenies et al. 2011).

The RLR signaling program further intersects with the inflammasome signaling pathway. Moore et al. has identified NLRX1 (Nod9), a member of the nucleotide-binding domain and leucine-rich-repeat-containing (NLR) family in the regulation of RLR signaling (Moore, Bergstralh et al. 2008). NLRX1 localizes to the outer membrane of the mitochondria, and its interactions with IPS-1 potently inhibits RLR-dependent IFN induction by disrupting IPS-1 interactions with signaling-active RLRs. Depletion of NLRX1 expression enhanced virus-induced signaling and decreased virus replication, confirming NLRX1 as a negative regulator of RLR-induced antiviral responses. In contrast, NLRC5, another member of the NOD-like protein family interacts with RIG-I and MDA5 but not IPS-1 to inhibit RLR-mediated IFN responses (Cui, Zhu et al. 2010). NLRC5 interaction with IKKα and IKKβ further blocked their phosphorylation and inhibited their NF-κB activating activities. Consistent with this finding, siRNA silencing of NLRC5 expression was able to enhance NF-κB transcriptional activity to drive IFN production and signaling of the antiviral response. Other studies have revealed membrane associated or mitochondria-interacting cofactors as RLR signaling regulators, including mitofusin 1 and 2 (Yasukawa, Oshiumi et al. 2009; Castanier, Garcin et al. 2010; Onoguchi, Onomoto et al. 2010; Koshiba, Yasukawa et al. 2011), various mitochondria outer membrane proteins, including TOM70 (Liu, Wei et al. 2010), and specific transmembrane proteins such as STING (otherwise known as MITA or MPYS) (Ishikawa and Barber 2008; Zhong, Yang et al. 2008). In the case of the mitofusins, they are thought to link endoplasmic reticulum with mitochondria to govern mitochondria dynamics that occur during virus infection, likely influencing IPS-1-dependent RLR signaling (Yasukawa, Oshiumi et al. 2009; Castanier, Garcin et al. 2010; Onoguchi, Onomoto et al. 2010; Koshiba, Yasukawa et al. 2011). STING has been identified as an RLR signaling cofactor and essential signaling adaptor protein that directs innate immune responses to DNA viruses (Ishikawa and Barber 2008; Zhong, Yang et al. 2008). Thus, its role in RLR signaling in RNA virus infection may offer overlap with the host response to DNA virus infection. These interactions of NLRs, mitofusins, STING, and other mitochondrial proteins with the RLR signaling pathway are likely to impose control of RLR signaling events that serve to program the pro-inflammatory response, though this idea has yet to be validated.

Positive and Negative regulation through differential ubiquitination and polyubiquitin binding

RLR signaling is tightly regulated by a number of mechanisms to prevent aberrant interferon production which may otherwise lead to immune toxicity (such as a “cytokine storm”) or the development of autoimmune disorders. Post-translational modifications such as ubiquitination or deubiquitination of key components of the RLR signaling pathway appears to be a major point of regulation. Riplet, otherwise known as RNF135 or REUL is an ubiquitin ligase that interacts with RIG-I but not MDA5 (Gao, Yang et al. 2009; Oshiumi, Matsumoto et al. 2009). It is reported to mediate the conjugation of K63-linked polyubiquitin chains to RIG-I within the CARD at aa K154, 164 and 172 (Gao, Yang et al. 2009) as well as within the RD (Oshiumi, Miyashita et al. 2010) and is essential for virus-induced IFN signaling. Mice deficient in RING finger protein leading to RIG-I activation (Riplet;otherwise known as RNF135 or REUL, which functions as a ubiquitin ligase) expression were defective in IFN and cytokine production during RNA virus infection and as a consequence were more susceptible to virus infection compared to wild type mice. TRIM25 also mediates K63-linked polyubiquitination of RIG-I at aa K172 during virus infection. This modification is thought to stabilize interactions between RIG-I and IPS-1 to induce signaling activation and IFN production (Gack, Shin et al. 2007; Gack, Kirchhofer et al. 2008). However, free K63-linked polyubiquitin chains are capable of inducing RIG-I activation in an *in vitro* reconstitution of the RIG-I pathway, suggesting that it is K172-mediated polyubiquitin-binding and not ubiquitin-modification that might drive RIG-I activation (Zeng, Sun et al. 2010). The data further suggests that K63-linked polyubiquitin chains act as a second ligand for RIG-I activation. This notion is also supported by the findings that i) expression of a K172R RIG-I mutant that cannot be polyubiquitinated was able to reconstitute Sendai virus-induced signaling to wildtype levels in RIG-I-deficient MEFs, and ii) the lysine 172 of RIG-I is not conserved between different species (Shigemoto, Kageyama et al. 2009). Nistal-Villan et al. recently reported the regulation of TRIM25 activities through the phosphorylation of RIG-I at specific residues (Nistal-Villan, Gack et al. 2010). RIG-I phosphorylation at serine 8 and or threonine 170 inhibited TRIM25-RIG-I interaction and ubiquitination of RIG-I. However, the regulation by phosphorylation of serine 8 is not likely to be universal as this aa residue is only shared among primate RIG-I and is not conserved among non-primate species. RIG-I signaling is also subject to regulation by the ubiquitin editing protein A20 (Lin, Yang et al. 2006). A20 has both deubiquitination and ubiquitin ligase activities. However, structure-function analyses reveal that only the ubiquitin ligase activity associated with its C-terminus domain is important for regulating RLR signaling. Overexpression of A20 selectively inhibited RIG-I-dependent activation of IRF3 and NF-κB whereas its depletion from cells enhanced virus induced signaling, suggesting that it functions as a negative regulator in the RLR pathway. In addition, TNFR associated factor 3 (TRAF3), a K63-linked E3 ubiquitin ligase that is essential for regulating virus-induced IRF3 activation (Hacker, Redecke et al. 2006; Oganesyan, Saha et al. 2006) was found to regulate IFN but not inflammatory cytokine production during virus infection. TRAF3 binds to the TRAF-interacting motif (TIM), which is found within the proline-rich region of IPS-1 and facilitates IKKε recruitment to the IPS-1 signalosome. TRAF3 activity in RLR signaling is further regulated by the E3 ubiquitin ligase Triad3A (Nakhaei, Mesplede et al. 2009), the deubiquitinase OTUB1 and OTUB2 (Li, Zheng et al. 2010), the deubiquitinase DUBA (Kayagaki, Phung et al. 2007), the interferon inducible gene FLN29 (Mashima, Saeki et al. 2005; Sanada, Takaesu et al. 2008), by the stability of IKKε interactions with IPS-1 (Paz, Vilasco et al. 2009; Paz, Vilasco et al. 2011) and by interactions between the Polo-like kinase 1 (PLK1) and IPS-1 (Vitour, Dabo et al. 2009). Thus, RLR signaling is regulated through multiple activities of ubiquitination ligase networks that operate alone or in concert. This complexity of signaling control may serve to tune the RLR response to specific stimuli.

RNF125 is another ubiquitin ligase that cooperates with the ubiquitin E2 ligase HbcH5c to conjugate K48-linked ubiquitin to RIG-I, MDA5 and IPS-1 to mediate proteasomal degradation. RNF125 itself is an ISG whose expression is induced following virus infection and its action is part of a negative feedback loop to prevent excessive interferon production (Arimoto, Takahashi et al. 2007). The activity of RNF125 is suppressed by UbcH8, the same ubiquitin ligase that is responsible for conjugating ISG15 to target proteins during virus infection (Arimoto, Konishi et al. 2008). Based on accumulating evidence, it is proposed that ISG15 interaction with UbcH8 concurrent with virus-induced ISG15 expression dissociates it from its interaction with RNF125. This action then would facilitate RNF125 conjugation of ubiquitin to RIG-I and other molecules to inhibit RLR signaling of IFN expression. Consistent with this model, basal expression of RIG-I is higher in Ube1-deficient cells that lack the ability to conjugate ISG15 as compared to wild type cells, thus providing RIG-I amounts that facilitate robust RLR signaling (Kim, Hwang et al. 2008). In addition, the tumor suppressor CYLD is a deubiquitinase that interacts with both RIG-I and IPS-1. It was previously shown to be essential in preventing aberrant IKKε-and TBK1 activation (Zhang, Wu et al. 2008). Consistent with this finding, cells deficient in CYLD expression signal activation constitutively and exhibit a hyperinduction of IFN during virus infection. Moreover, a recent study suggests that CYLD functions to remove polyubiquitin chains from RIG-I and TBK1 to inhibit IRF-3 signaling and RIG-I dependent IFN production from the IPS-1 signalosome (Friedman, O'Donnell et al. 2008). These studies define a critical role for the reversible ubiquitination of RLRs and cofactors within the RLR signaling pathway in the regulation of RLR pathway signaling during the immune response to virus infection.

Post-translational control of RLRs: phosphorylation, other modifications, and protein interactions

The RLR signaling pathway is further regulated by additional post-translational modifications, including the phosphorylation, acetylation and SUMOylation of signaling components. Beyond the modification events that govern ubiquitination described previously, post-translational modification of RIG-I may further serve to prevent premature activation in the absence of PAMP and represents another level of regulation of RLR activation. For instance, casein kinase II phosphorylates RIG-I in its resting state at aa residues threonine 770, serine 854 and serine 855 (Sun, Ren et al. 2011). Mutation of these sites, chemical inhibition or depletion of casein kinase II rendered RIG-I constitutively active resulting in enhanced IFN induction. In contrast, the treatment of cells with phosphatase inhibitor suppressed RLR-dependent signaling, suggesting that phosphorylation of RIG-I is required to maintain autoregulation by its RD. Additionally, RIG-I was identified by mass spectrophotometry to be acetylated at amino acid residues K858 and K909 however it remains to be determined how lysine acetylation regulates RIG-I signaling activity (Choudhary, Kumar et al. 2009).

Downstream signaling components involved in RLR signaling are also regulated by post-translational modifications. Of note is that virus-induced activation of TLR and RLR pathways is known to lead to the SUMOylation of IRF3 and IRF7 at K152 and K406 respectively. Mutants of these factors that do not support SUMO-modification exhibit enhanced IFN induction after viral infection suggesting that SUMO-modification of IRF3 and IRF7 is a negative regulatory step in RLR signaling (Kubota, Matsuoka et al. 2008). Thus, specific modification of RLRs and their signaling cofactors impose regulation of innate defense signaling at a variety of levels ranging from RLR activity to IPS-1 signalosome assembly and function.

Interactions with regulatory proteins further serve as points of control in RLR-dependent signaling. Primary to this is the interaction of RIG-I or MDA5 directly with IPS-1 to drive

IPS-1 signalosome assembly and/or activation. Further, interaction of IPS-1 signalosome components with regulator factors serves to modulate RLR signaling actions through IPS-1. For example, SIKE is a physiological suppressor of TBK1 and IKKε that keeps these protein kinases sequestered as inactive complexes to prevent unintended activation by either RLRs (Huang, Liu et al. 2005). Moreover, the Atg5-Atg12 conjugate previously implicated in the induction of autophagic responses directly associates with RIG-I and IPS-1 CARDs to suppress virus-induced signaling (Jounai, Takeshita et al. 2007; Takeshita, Kobiyama et al. 2008). PSMA7 or the proteasome alpha4 subunit associates with IPS-1 to inhibit virus-induced RLR-mediated signaling, although it is unclear how this is accomplished (Jia, Song et al. 2009). Additionally, the tyrosine kinase c-Src interacts with IPS-1, TBK1 and TRAF3, likely within the IPS-1 signalosome, to enhance RLR-dependent IFN induction (Johnsen, Nguyen et al. 2009). Separately, the Src-like non-receptor protein kinase, c-Abl has been shown to interact with and phosphorylate IPS-1 during virus infection to facilitate induction of signaling to both IRF-3 and NF-κB (Song, Wei et al. 2010). Furthermore, Eyes absent 4 (EYA4) and its phosphothreonine-specific phosphatase activity is required to facilitate RLR-mediated IFN signaling (Okabe, Sano et al. 2009). EYA4 was reported to interact with IPS-1, STING and NLRX1, again most likely within the IPS-1 signalosome. Finally, dihydroxyacetone kinase (DAK) selectively interacts with the MDA5 CARD to regulate MDA5-mediated antiviral signaling, likely by altering the MDA5-IPS-1 interaction (Diao, Li et al. 2007). It is speculated that DAK sequesters MDA5 in an inactive state but releases MDA5 from such regulation during virus infection wherein it can bind to and modulate the actions of IPS-1. Taken together, the studies reviewed above demonstrate that the RLR pathway and the activity of its components are subject to regulation through direct protein interaction and/or post-translational modifications that act in concert to mediate RLR-induced IFN production and the expression and immune-response genes for the control virus infection.

RLRs cooperate with other PRRs in the detection of viruses

During virus infection RLRs do not operate along to induce and program antiviral immunity but part of a concerted and crosstalking antiviral program mediated by a variety of PRRs. An example if how the RLRs and other PRRs interact to participate in immune signaling during virus infection is well-illustrated through in vivo studies of West Nile virus (WNV) infection (a flavivirus) in mice. Genetic studies using knockout mice show that a close cooperation between PRRs is important in triggering the initial innate immune response and in defining the quality of the adaptive immune response to limit WNV dissemination and neuroinvasion. WNV infection of a mammalian host is initiated by the bite of a an infected mosquito and has been effectively modeled in mice. The insect to mammal host infection event first transmits the virus to resident langerhans cells in the skin and also likely skin fibroblasts. The infection stimulates local IFN production and innate immune defenses through essential RLR signaling within the infected cells and serves to suppress peripheral spread of the virus. However, after an initial round of replication at the skin portal of entry the virus disseminates to the draining lymphnode where it undergoes further amplification in resident macrophages and DCs where it is engaged by RLRs and TLRs to drive diversified IFN and proinflammatory cytokine production, thus inducing a local inflammatory response. While RLR signaling is essential for sensing WNV infection and for the initial triggering of the intracellular innate immune response, the TLRs serve a secondary role this capacity to drive specific cytokine production leading to regulation of the adaptive immune response and programming of cell mediated immunity (Daffis, Suthar et al. 2009; Suthar, Ma et al. 2010). It should be noted that the expression of TLRs and many of their signaling cofactors is induced and enhanced by IFN (Hall and Rosen 2010). Thus, initial RLR signaling and IFN production against virus infection serves to enhance TLR expression and to potentiate the actions of the TLR signaling pathways. In this capacity the RLRs provide signaling

crosstalk to enhance TLR expression and function, leading to global immune modulation that plays a role in controlling the peripheral dissemination of WNV infection among tissues. At this point of the infection WNV is carried to the spleen via processes of infected cell migration and viremia, where the resident cells initiate RLR dependent innate immune actions after virus exposure. If WNV can overcome or evade these antiviral actions it will further replicate and invade the central nervous system. resulting in encephalitis and possibly death.

RIG-I and MDA5 are both important for controlling WNV infection. Although RIG-I deficient cells are able to mount an IFN-dependent antiviral response, this response is attenuated and delayed in its onset compared to wild type cells (Fredericksen and Gale 2006; Fredericksen, Keller et al. 2008). In contrast, antiviral signaling is abrogated in cells lacking both RIG-I and MDA5 or their common adaptor IPS-1. Correspondingly, the cells lacking these factors support enhanced virus replication as compared to wild type cells, suggesting that RIG-I and MDA5 cooperate to direct the innate immune response and the amplification of IFN signaling to control WNV infection. Innate immune signaling against other flaviviruses show a similar dependence on RIG-I and MDA5 (Kato, Takeuchi et al. 2006; Loo, Fornek et al. 2008). This finding is further supported by ex vivo studies showing that innate immune signaling is abrogated in cellslacking IPS-1 and able to mediate RLR signaling (Fredericksen, Keller et al. 2008; Daffis, Suthar et al. 2009; Suthar, Ma et al. 2010). Moreover, IPS-1 deficient mice exhibit severe dysregulation of both innate and adaptive immune processes that fail to contain the infection, resulting in neuroinvasion and increased susceptibility of mice to WNV infection (Daffis, Suthar et al. 2009; Suthar, Ma et al. 2010). Signaling crosstalk to the RLRs from caspase 12-medaited pathways of the infected cells has been shown to enhance TRIM25-mediated ubiquitination of RIG-I, thus potentiating RLR signaling. Accordingly, caspase 12 deficient mice exhibit increased viral burden after WNV infection (Wang, Arjona et al. 2010). These findings suggest that caspase 12 may provide signaling crosstalk that regulates RIG-I activity in the control of virus infection.

Enhancement of TLR expression in response to RLR signaling impacts MyD88 dependent signaling that controls cell-type susceptibility to WNV infection (Welte, Reagan et al. 2009; Szretter, Daffis et al. 2010). Although there was little difference reported in in the systemic IFN response to WNV infection, MyD88 deficient mice were shown to be more more susceptible to WNV infection than wild type counterparts and they exhibit increased peripheral spread of the virus with high viral load in the central nervous system, with reduced levels of leukocyte trafficking into the brain (Szretter, Daffis et al. 2010). These observations indicate that RLR signaling crosstalk with MyD88 dependent TLR signaling imparts control of WNV replication in neuronal cells.

While RLRs crosstalk with TLRs to diversify the innate immune response to virus infection, this crosstalk can also have a pathogenic outcome. Indeed, TLR3 deficient mice have been shown have improved survival rates to WNV infection as compared to wild type controls (Wang, Town et al. 2004). The increased survival was attributed to a decrease in the inflammatory response that was mediating WNV penetration of the blood brain barrier. In this case crosstalk of RLR signaling to enhance TLR3 expression and pathway signaling was likely a contributor to the pathogenic outcome. This idea is supported by studies of mice lacking SARM, an adaptor protein that negatively regulates TLR3. SARM deficient mice exhibited increased lethality after WNV challenge that was associated with increased virus in the central nervous system, implicating the RLR to TLR3 crosstalk and TLR3 function in the pathology of WNV infection(Szretter, Samuel et al. 2009). However, separate studies indicate that TLR3 and its crosstalk with RLRs impart protection from virus replication and WNV dissemination into the central nervous system in both mice and humans (Kong,

Delroux et al. 2008;Daffis, Samuel et al. 2008); RLR signaling to enhance TLR7 expression may also serve to control WNV infection but through immune cell modulation in which TLR7 imparts immune cell homoing to infected tissues (Town, Bai et al. 2009), and likely in a cell specific or context-dependent manner (Welte, Reagan et al. 2009).Thus, RLR signaling crosstalk to TLRs serves to enhance TLR signaling programs for the regulation of innate immune effector actions that control virus replication within infected cells and that suppress virus spread and dissemination in vivo while modulating immune cell trafficking and functions that suppress infection in tissues.

RLR regulation of the adaptive immune response

In addition to its role in driving innate immune defenses, IFN plays a major role in modulating the adaptive immune response. IFN is required to promote T cell survival and clonal expansion after antigen presentation (Marrack, Kappler et al. 1999; Curtsinger, Valenzuela et al. 2005; Kolumam, Thomas et al. 2005). Moreover, interferon potently induces the cytolytic activity of natural killer cells and cytotoxic lymphocytes (Biron, Nguyen et al. 1999; Curtsinger, Valenzuela et al. 2005), and plays an important role in promoting B cell differentiation and antibody production (Le Bon, Schiavoni et al. 2001; Jego, Palucka et al. 2003). Interferon actions further facilitate antigen presentation processes by promoting the expression of MHC class I molecules on most cell types and co-stimulatory molecules on antigen presenting cells (Stark, Kerr et al. 1998). However, the specific role of RLR signaling in regulating interferon production and its regulation of the adaptive immune response is less clear, and appears to vary from virus to virus.

Results showing that RLR signaling imposes modulation of adaptive immunity are beginning to reveal how RLRs program the immune response. As an example, mice that received an influenza virus DNA-based vaccine co-expressing a RIG-I agonist that activated RLR signaling exhibited increased virus-specific serum antibody response as compared to those that were provided with the DNA vaccine alone (Luke, Simon et al. 2011). While these results suggest that RLR signaling can enhance antibody development driven by vaccine administration, they contrast somewhat to a study that compared TLR and RLR signaling of adaptive immunity. In this case when infected with influenza A virus, mice lacking MyD88 and thereby unable to signal through most TLRs, failed to induce antigen-specific B and T cell activation but by comparison mice lacking IPS-1 and unable to mediate RLR signaling showed no such defect (Koyama, Ishii et al. 2007). However, and in contrast to this study, mice lacking IPS-1 when infected with West Nile virus (a flavivirus) exhibited elevated systemic IFN, proinflammatory cytokine and chemokine levels, increased but dysregulated B and T cell activation, loss of neutralizing antibodies despite higher overall antibody production, and a general failure to protect the mice from infection (Suthar, Ma et al. 2010). This study also showed that T regulatory cells failed to expand during acute infection of mice lacking IPS-1 that are therefore unable to signal through RLR pathways. Moreover, Anz et al. further observed that both T effector and T regulatory cells express RIG-I and MDA5, and that RLR-signaling is required for encephalomyocarditis virus (a picornavirus)-induced regulation of T regulatory cell function during infection (Anz, Koelzer et al. 2010). In a separate study, IPS-1 was found to be essential for the innate immune but not the cytotoxic T lymphocyte response in mice during respiratory syncytial virus (a paramyxovirus) infection (Bhoj, Sun et al. 2008). The results suggest that at in the case of flavivirus and picornavirus infections, RLR signaling is important in the control of the quality, quantity, and balance of the adaptive immune response during infection, while RLR regulation of the adaptive immune response is likely to be specific and differential for specific virus infections.

RLR polymorphisms and immune disease

Accumulating evidence suggest that aberrant IFN induction and the resulting signaling of innate immune programs is associated with autoimmune disease (reviewed by (Hall and Rosen 2010)). As receptors that regulate IFN induction, there is increasing interest in assessing potential links between RLR function and and/or polymorphisms that may lead to differences in susceptibility to infection and autoimmune diseases. Genetic screens have led to the identification of a number of polymorphisms in *IFIH1* (the MDA5 gene) that are associated with resistance to type 1 diabetes (T1D), including: T946A, E627*, I923V, R843H, IVS8+1, and IVS14+1 (Smyth, Cooper et al. 2006; Concannon, Onengut-Gumuscu et al. 2008; Liu, Wang et al. 2009; Nejentsev, Walker et al. 2009). The polymorphisms are associated with i) reduced *IFIH1* transcription (Liu, Wang et al. 2009; Downes, Pekalski et al. 2010), ii) disruption of conserved splice donor sites leading to the expression of non-functional MDA5 variants (Nejentsev, Walker et al. 2009), and/or iii) expression of truncated or mutated variants of MDA5 that lead to defective RNA-binding and or IFN induction signaling (Shigemoto, Kageyama et al. 2009). It is also noted that accumulating evidence reveals the presence of picornavirus RNA and viral antigens in the pancreas, pancreatic islets and PBMCs of T1D patients as compared to healthy controls or patients with type 2 diabetes (Ylipaasto, Klingel et al. 2004; Williams, Oikarinen et al. 2006; Dotta, Censini et al. 2007; Zanone, Favaro et al. 2007; Richardson, Willcox et al. 2009). These studies suggest that aberrant MDA5-mediated IFN induction during picornavirus infection of pancreatic cells may underlie islet cell damage and the onset of T1D. Consistent with this idea, mice lacking one copy of MDA5 developed transient hyperglycemia after infection with beta cell-tropic encephalomyocarditis virus-D, a beta cell tropic virus (McCartney, Vermi et al. 2011). Moreover, silencing of MDA5 expression in rat pancreatic beta cells by siRNA treatment reduced poly(I:C) induced expression of proinflammatory cytokine and IFN (Colli, Moore et al. 2010), suggesting that aberrant interferon induction by MDA5 could be a factor in beta cell programming of T1D. In addition to its linkage with T1D, MDA5 was recently identified as the 140kDa autoantigen most frequently associated with clinically amyopathic dermatocyositis (Betteridge, Gunawardena et al. 2009; Sato, Hoshino et al. 2009; Nakashima, Imura et al. 2010). Subjects that exhibit an accumulation of autoantibodies to MDA5 are often associated with a higher frequency of rapidly progressive interstitial lung disease (Sato, Hirakata et al. 2005; Gono, Kawaguchi et al. 2010; Nakashima, Imura et al. 2010).

The evidence implicating RIG-I involvement in autoimmune disease is less clear. One report shows that subjects with Crohn's disease but not ulcerative colitis exhibit a selective suppression of RIG-I expression in the intestinal epithelial compartment (Funke, Lasitschka et al. 2011). In another report, RIG-I-deficient mice generated by the deletion of exons 4-8 spontaneously develop a phenotype characteristic of autoimmune colitis. The phenotype included inflammation and damage of the colon mucosa, reduction in number and size of Peyer's patches, and suppression of Gαi2 expression (Wang, Zhang et al. 2007). Together, these studies suggest a link between aberrant RIG-I expression and autoimmune diseases involving the gut.

Polymorphisms leading to the expression of alternate RIG-I variants have also been reported. One common, non-synonymous polymorphism leads to the expression of a functional RIG-I with an arginine to cysteine mutation at aa position 7 (Shigemoto, Kageyama et al. 2009) that is associated with increased antiviral signaling (Hu, Nistal-Villan et al. 2010). This same polymorphism was shown to be associated with a decrease in humoral immunity development in children who were given the rubella vaccine (Ovsyannikova, Haralambieva et al. 2010). Peripheral blood mononuclear cells (PBMCs) from patients who exhibit a second polymorphism resulting in the expression of a non-

functional variant of RIG-I encoding a serine to isoleucine mutation at aa position 183 were severely attenuated in antiviral signaling against influenza A virus and Sendai virus (Pothlichet, Burtey et al. 2009). Furthermore, a loss-of-function polymorphism of IPS-1 that led to the expression of a non-functional IPS-1 variant expected to abrogate RLR signaling has been linked to a subtype of systemic lupus erythematosus (Pothlichet, Niewold et al. 2011). Taken together, the studies suggest that dysregulation of RLR signaling programs may lead to the development of autoimmune diseases, and polymorphisms in the RLR genes and their signaling components may define susceptibility to virus infection.

Conclusion

RLRs are essential pathogen recognition receptors that impart recognition of RNA virus infection. RLR signaling programs rely on the IPS-1 adaptor protein and its assembly of a high energy signalosome that drives downstream activation of transcriptional responses that induce interferon and antiviral and immune modulatory genes that control virus replication and spread, and that serve to regulate the adaptive immune response. While RLR signaling activation by ligand interaction serves to initiate the immune response to virus infection, an increased understanding of the molecular features underlying these processes could offer new strategies to consider for immune and antiviral therapy by targeting the RLR pathway for the therapeutic control of virus infection, enhancement of the immune response, and even for strategies of immune suppression to control inflammation or specific autoimmune diseases.

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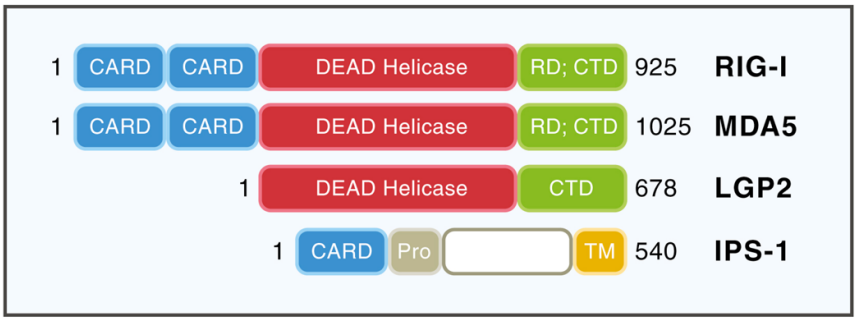

Figure 1. Structural representation of the RLRs and their adaptor IPS-1

Key structural domains involved in signaling are shown. The RLRs consists of CARD (caspase activation and recruitment domain); ATPase containing DEAD box helicase (DEAD helicase) and a C-terminal domain (CTD) that in RIG-I and LGP2 but not MDA5 encodes a repressor domain (RD) involved in autoregulation. LGP2 lacks the N-terminal CARDs. IPS-1 consists of a homologous CARD, a proline-rich region (Pro), and a transmembrane domain (TM) on its C-terminus.

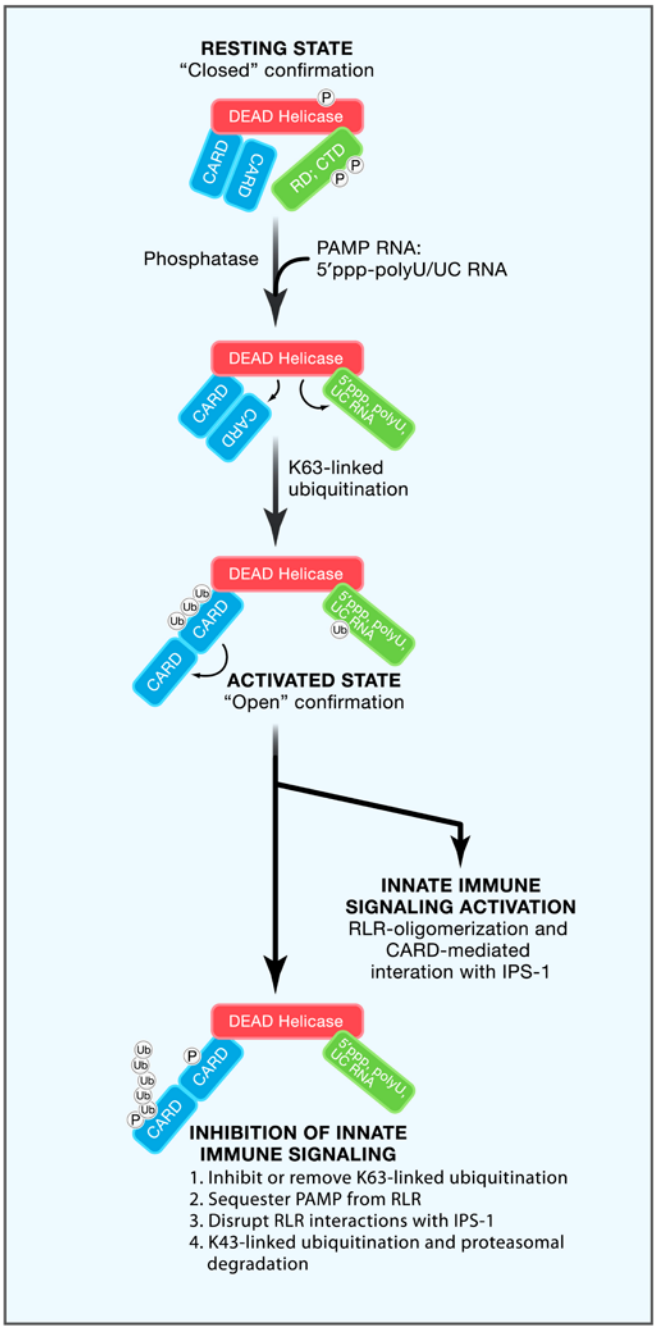

Figure 2. Model for RIG-I signaling activation and its regulation

In the resting state, RIG-I is held in a "closed" conformation in the resting state by casein kinase II phosphorylation and autoregulatory intramolecular interactions with the C-terminal RD within the CTD holds the CARDs unavailable for signaling. During virus infection, RD engagement of a 5′ppp RNA PAMP containing and other nonself signature(s), concommitant with K63-linked ubiquitination of RIG-I by TRIM25 and RING finger protein leading to RIG-I activation (Ripletotherwise known as REUL or RNF135), leads to conformational changes that release the CARDs from autoregulation. The model shows RIG-I binding to 5′ppp RNA containing poly-U/UCmotif such as the HCV RNA genome (Saito, Owen et al. 2008; Uzri and Gehrke 2009). RIG-I then assumes an "open"

conformation that allows for oligomerization and CARD-dependent interaction with IPS-1 which activates signaling molecules at the IPS-1 singalosome. These interactions trigger a signaling cascade that culminates in IFN production and expression of proteins with direct antiviral or immune-modulating activities to control infection. To prevent excessive activation of innate immune responses, RIG-I signaling activity is inhibited by (1) phosphorylation events that inhibit the K63-linked ubiquitination required for signaling activation, (2) negative regulators that sequester PAMP from RIG-I, (3) association with negative regulators or molecules that disrupt/destabilize its interaction with IPS-1, and (4) K43-linked ubiquitination by RNF125 which targets RIG-I for proteasomal degradation.

Table 1
RLR detection of viral genera and viral RNA genome type

Viruses detected by RIG-I
Paramyxoviridae (-) ssRNA, NS
Sendai virus (Kato, Sato et al. 2005; Yoneyama, Kikuchi et al. 2005)
Newcastle disease virus (Kato, Sato et al. 2005)
Respiratory syncytial virus (Loo, Fornek et al. 2008)
Measles (Plumet, Herschke et al. 2007)
Nipah (Habjan, Andersson et al. 2008)
Human parainfluenza 5 mRNA (Luthra, Sun et al. 2011)
Rhabdoviridae (-) ssRNA, NS
Vesicular stomatitis virus (Kato, Sato et al. 2005; Yoneyama, Kikuchi et al. 2005)
Rabies virus (Hornung, Ellegast et al. 2006)
Orthomyxoviridae (-) ssRNA NS
Influenza A (Kato, Takeuchi et al. 2006)
Influenza B (Loo, Fornek et al. 2008)
Filoviridae (-) ssRNA NS
Ebola (Habjan, Andersson et al. 2008)
Arenaviridae (-) ssRNA S
Lassa (Habjan, Andersson et al. 2008)
Lymphocytic choriomeningitis virus (Zhou, Cerny et al. 2010)
Bunyaviridae (-) ssRNA S
Rift Valley fever virus (Habjan, Andersson et al. 2008)
Flaviviridae (+) ssRNA NS
Hepatitis C virus (Sumpter, Loo et al. 2005; Saito, Hirai et al. 2007)
Coronaviridae (+) ssRNA NS
Murine hepatitis virus (Roth-Cross, Bender et al. 2008)
Caliciviridae (+) ssRNA NS
Murine norovirus-1 (McCartney, Thackray et al. 2008)
DNA viruses
Epstein-Barr virus EBER (Samanta, Iwakiri et al. 2006)
Myxoma virus (Wang, Gao et al. 2008)
Viruses detected by MDA5
Picornaviridae (+) ssRNA NS
Encephalomyocarditis virus (Gitlin, Barchet et al. 2006; Kato, Takeuchi et al. 2006)
Theiler's virus (Kato, Takeuchi et al. 2006)

Mengo virus (Kato, Takeuchi et al. 2006)
DNA viruses
Vaccinia virus (Pichlmair, Schulz et al. 2009)
Flaviviruses detected by both MDA5 and RIG-I
Flaviviridae
Japanese encephalitis virus (Kato, Takeuchi et al. 2006)
Dengue virus (Loo, Fornek et al. 2008)
West Nile virus (Fredericksen, Keller et al. 2008)
Reoviridae dsRNA S
Reovirus (Kato, Takeuchi et al. 2008; Loo, Fornek et al. 2008)

Annotations regarding the virus genome include the nucleotide composition e.g. single-stranded (ssRNA) or double-stranded RNA (dsRNA); positive (+) or negative (-) sense genomic orientation, and segmentation (S) versus non-segmentation (NS).