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Cytoplasmic Viral RNA Sensors: RIG-I-Like Receptors

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Abstract

The interferon (IFN) response is a powerful system that was evolutionarily acquired by vertebrates including mammals to protect against viral infection. The cytoplasmic RNA helicases, RIG-I-like receptors (RLRs), were discovered in 2004 as viral sensors that trigger the antiviral IFN response by recognizing the nonself signatures of viral RNAs. The mechanisms underlying the recognition of viral RNAs and signal transduction leading to the production of type I IFN have been intensively studied following the discovery of RLRs. Moreover, a dysregulation in the expression of RLR or aberrant RLR signaling has been implicated in the development of a number of autoimmune diseases. We herein provide an overview of recent advances in RLR research and discussed future directions.

Discovery of RIG-I-Like Receptors and Their Domain Structure

Recent findings have demonstrated that three major systems: Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and DNA sensors, including cGAS and IFI16, function as key sensors that recognize viral nucleic acids and trigger the induction of type I interferon (IFN) in the human body (Kawai and Akira, 2011; Yoneyama et al., 2015; Cai et al., 2014; Jakobsen and Paludan, 2014). The transfection of exogenous double-stranded RNA (dsRNA), typically the synthetic dsRNA, poly I:poly C, is known to induce IFN in a TLR-independent fashion. Therefore, cytoplasmic viral RNA sensors had been proposed to detect invading and replicating viruses. The cytoplasmic RNA helicase RIG-I was identified in 2004 as a viral sensor belonging to a family of three RLR members: RIG-I, MDA5, and LGP2 (Yoneyama et al., 2004, 2005; Rothenfusser et al., 2005). A fragment containing the N-terminus portion of RIG-I was identified by expression cloning and found to contain two tandem caspase activation and recruitment domains (CARDs), essential domains for signal transduction (Yoneyama et al., 2004). RLRs are located in the cytoplasm, and their expression is basally sustained at a low level. Upon infection, RLRs trigger antiviral IFN responses by the recognition of viral RNAs, and the IFN produced highly upregulates the expression of RLRs for the efficient sensing and amplification of antiviral signaling.

Structural analyses, including crystallographic studies, of these helicases revealed that RIG-I and MDA5 were composed of three main structural domains as shown in Figure 1: the CARD at the N-terminus for signal transduction, central DExD/H-box RNA helicase domain with RNA-dependent ATP hydrolysis activities (ATPase), and C-terminal domain (CTD) for RNA binding (Takahashi et al., 2008; Cui et al., 2008; Wang et al., 2010). This helicase domain is also known to have the potential to unwind dsRNA; however, RNA unwinding per se does not transduce signaling (Marques et al., 2006), instead its ATP hydrolysis has been implicated in conformational changes associated with dsRNA binding (Jiang et al., 2011). Detailed structural studies of RIG-I have

recently revealed that a central DExD/H-box core is comprised of two RecA-like folds, designated Hel-1 and Hel-2, with the latter containing an insertion of Hel-2i that facilitates the recognition of dsRNA (Kowalinski et al., 2011; Jiang et al., 2011; Luo et al., 2011; Civril et al., 2011). Recent studies also identified a novel Pincer domain (also designated as a bridging domain) composed of two alpha helices physically tethering the CTD to the helicase core (Kowalinski et al., 2011; Luo et al., 2011). In spite of its pronounced influence on the structure of RIG-I, few studies have examined the functional significance of the Pincer region with regard to ligand binding, allosteric regulation, and helicase and ATPase activities as well as the signaling activities of RIG-I (Kageyama et al., 2011).

The third member, LGP2 consists of a helicase domain and CTD (Figure 1). Although LGP2 lacks the CARD for its own signal transduction, a loss-of-function analysis revealed that it acted as a positive regulator of RLR signaling (Satoh et al., 2010). The importance of the ATPase activity of LGP2 in positive regulation was also demonstrated using knock-in mice with the LGP2 gene without ATPase activity (Satoh et al., 2010). The mechanisms underlying positive regulation have been suggested to increase the affinities of RIG-I and MDA5 to their ligands, dsRNA (Bruns et al., 2014; Pippig et al., 2009); however, the precise mechanisms have not yet been determined.

Recognition of Viral RNA by RLRs

RLR is expressed ubiquitously and promotes antiviral responses upon viral infection. Loss-of-function experiments revealed that RIG-I and MDA5 sensed a different spectrum of viral infections *in vivo* and in cell cultures, as summarized in Figure 2 (Kato et al., 2005, 2006; Gitlin et al., 2006). For example, RIG-I was found to be essential for the recognition of various types of RNA viruses, including influenza A virus (IAV), hepatitis C virus (HCV), Sendai virus, and vesicular stomatitis virus, whereas MDA5 preferentially recognized specific types of RNA viruses, including

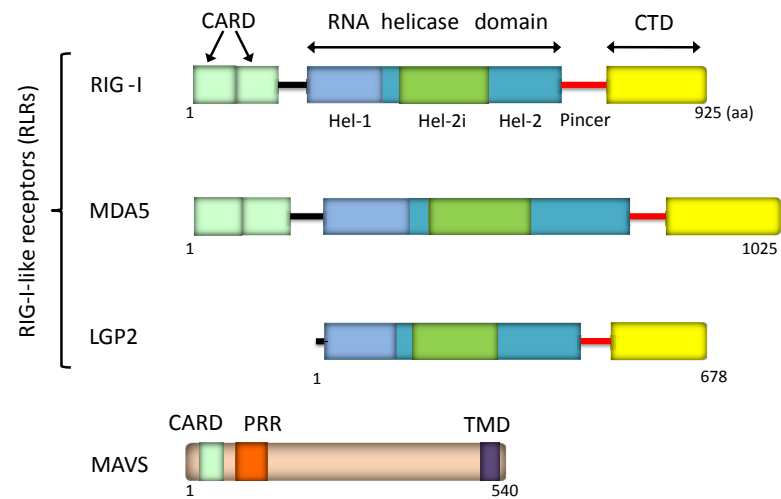


Figure 1 Domain structure of RIG-I-like receptors (RLRs) and MAVS. RIG-I and MDA5 are composed of three main structural domains: the tandem caspase activation and recruitment domains (CARDs) at the N-terminus for signal transduction, central DExD/H-box RNA helicase domain (Hel-1, Hel-2i, and Hel-2) with RNA-dependent ATP hydrolysis activities, and C-terminal domain (CTD) for RNA binding. LGP2 consists of a helicase domain and CTD. Crystal structure identified Pincer (red bar), which tethers the CTD to the helicase core. MAVS is composed of single CARD, a proline-rich region (PRR), and transmembrane domain (TMD).

Sensor	Repertoire of viruses detected by the sensor	RNA ligands
RIG-I	Influenza A virus (IAV) Hepatitis C virus (HCV) Sendai virus Newcastle disease virus Vesicular stomatitis virus Japanese encephalitis virus Respiratory syncytial virus West Nile virus Reovirus Rota virus	dsRNA(< 2 kbp) 5'ppp-dsRNA, 5'pp-dsRNA (Reovirus etc) Panhandle region of viral genome RNA polIII-derived RNA poly-U/UC rich RNA RNaseL-cleaved RNAs
MDA5	<u>Picornavirus</u> Mengovirus Encephalomyocarditis virus Theiler's virus Coxsackie B virus West Nile virus Reovirus Rotavirus	dsRNA(> 2 kbp) 5'pp-dsRNA (Reovirus etc) RNaseL-cleaved self RNAs RNA web

Figure 2 Differential recognition of RNA viruses by RIG-I and MDA5. RIG-I and MDA5 sense a different spectrum of viral infections *in vivo* and in cell cultures. This differential recognition by RIG-I and MDA5 is mostly attributed to the distinct RNA structures of RIG-I-like receptors ligands produced by different viruses. RNA ligands of RIG-I and MDA5 are also indicated above.

encephalomyocarditis virus (EMCV), Theiler's virus, and the mengovirus of Picornaviridae (Kato et al., 2005, 2006; Sumpter et al., 2005). Some viruses including West Nile virus, dengue virus, and reovirus are recognized by both RIG-I and MDA5 (Fredericksen et al., 2008; Kato et al., 2008; Loo et al., 2008). This differential recognition by RIG-I and MDA5 has mostly been attributed to the distinct RNA structures produced by different viruses (Figure 2; Kato et al., 2006; Hornung et al., 2006; Pichlmair et al., 2006;

Rehwinkel et al., 2010). For example, the genomic RNA of IAV, which contains a 5'ppp- and dsRNA-containing panhandle structure, was shown to efficiently activate RIG-I (Rehwinkel et al., 2010; Pichlmair et al., 2006). In the case of EMCV infection, long dsRNAs, generated as replication intermediates, strongly activated MDA5 (Kato et al., 2008). The short dsRNA (~2 kbp) of the reovirus genome with 5'pp was previously reported to activate RIG-I, whereas dsRNAs over 2 kbp of the reovirus activated MDA5 (Kato et al.,

2008; Goubau et al., 2014). A high-order RNA structure is also known to be a requirement for the activation of MDA5 (Pichlmair et al., 2009). Host mRNA and tRNA both lack 5'ppp- or 5'pp signatures and the long dsRNA stretch, and, thus, escape detection by RLR, which indicates the significance of the 5' signature and/or length of dsRNA for the activation of RLR (Schlee et al., 2009; Kato et al., 2008). Sequence specificity for the activation of RLR such as the uridine-rich region in the 3'UTR of HCV genome RNA has also been suggested (Saito et al., 2008). In some cases, RLR ligands were found to be produced by host enzymes including polymerase III and RNase L. Previous studies demonstrated that the artificial transfection of AT dsDNA triggered RIG-I-dependent IFN signaling and also that AT dsDNA was transcribed by RNA polymerase III to generate 5'ppp-AU RNA (Ablasser et al., 2009; Chiu et al., 2009). However, its physiological significance in viral infection is not well established. Small RNAs produced from the cleavage of both viral and host RNAs by RNase L have been

implicated as ligands for RIG-I and MDA5 (Malathi et al., 2007). These cleavage products were shown to possess 5' OH and 3' monophosphate, and the latter was shown to be critical for the activation of RLR (Malathi et al., 2010).

Although the molecular features of RLR ligands have been reported, (1) the types of RNA produced by different viral infections, (2) the RNA molecules of viral (or host) products responsible for the activation of RLR under physiological settings, and (3) the exact molecular features of RLR-activating viral (or host) RNAs have yet to be determined.

Current Model for RLR Activation

The production of IFN is generally tightly regulated: IFN is undetectable in uninfected cells and is rapidly induced upon infection. Multiple steps have been proposed for the activation of RLR to trigger signaling, which ultimately leads to the production of IFN (Figure 3).

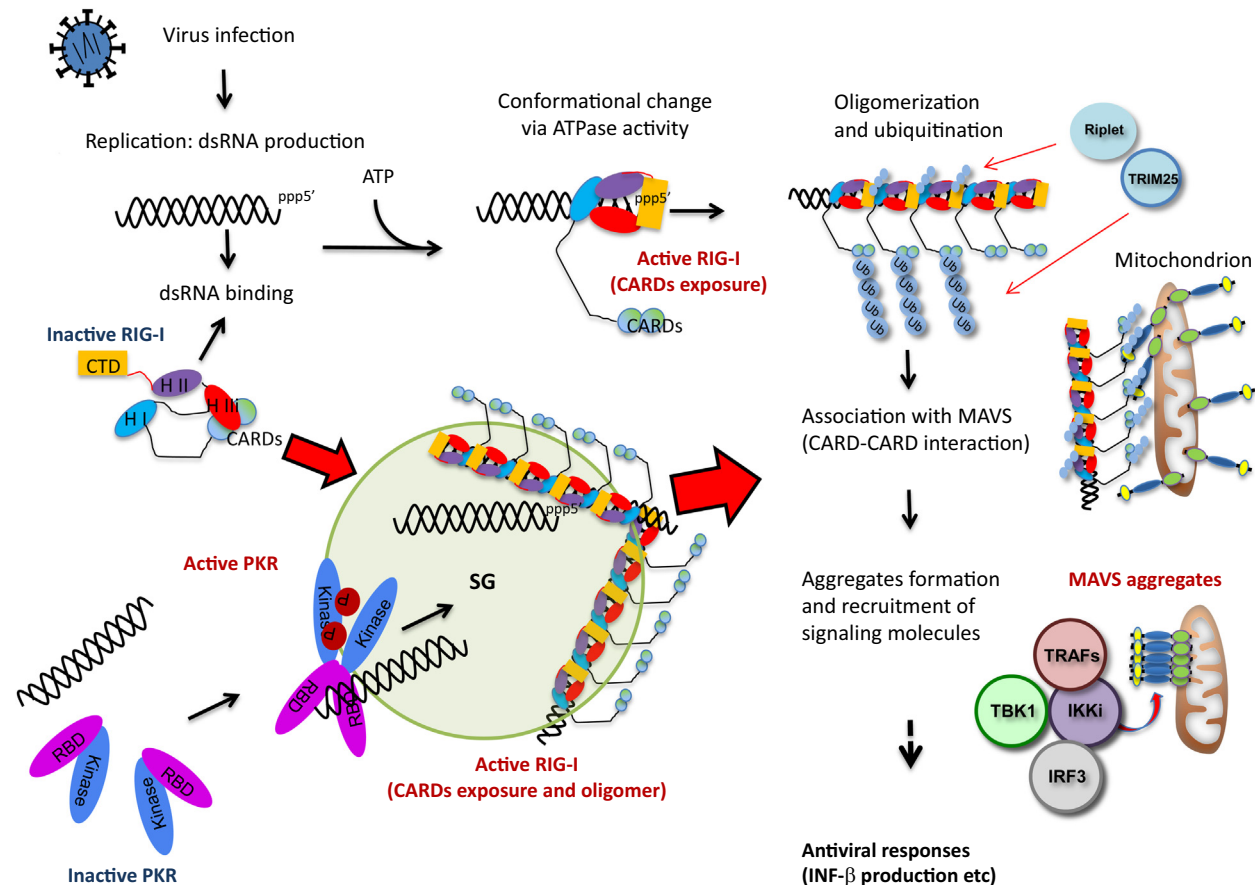


Figure 3 Activation steps of RIG-I in the cytoplasm. The first step involves a physical association between viral RNA and RIG-I. RIG-I undergoes a prominent conformational change: the C-terminal domain (CTD) binds tightly to 5'ppp or the blunt ends of 5' OH dsRNA and the helicase domains (Hel-I, Hel-II, Hel-III) wrap around dsRNA, leading to a more compact composition. These conformational alterations via ATPase activity in RIG-I-like receptors (RLRs) result in the exposure of caspase activation and recruitment domains (CARD) and lead an oligomerization of RIG-I. K63-linked polyubiquitination by TRIM25 and Riplet promotes and stabilizes the oligomerization. The exposure of oligomerized CARDs leads to a signal relay to another CARD-containing protein, MAVS via CARD-CARD interactions. The aggregate formation of MAVS recruits signaling molecules and finally induces the production of type I interferons (IFNs). In addition, viral dsRNA also activates PKR, leading to the phosphorylation of eIF2 α , which ultimately induces the formation of stress granules (SGs), containing SG components, RLRs, and viral RNAs, to promote the activation of RLR.

The first step involves a physical association between viral RNA and RLRs. In this direct binding, RIG-I undergoes a prominent conformational change: the CTD binds tightly to 5'ppp or the blunt ends of 5' OH dsRNA and the helicase domains (Hel-I, Hel-II, Hel-III) wrap around dsRNA, leading to a more compact composition (Takahasi et al., 2008; Wang et al., 2010; Lu et al., 2010, 2011). In spite of having a similar domain structure to that of RIG-I, MDA5 has been identified as a crystal structure that binds to dsRNA in a somewhat different manner. The helicase domain of MDA5 wraps around dsRNA and the CTD also forms a contact with Hel-I; thus, MDA5 forms a closed ringlike structure around dsRNA. These conformational alterations in RLRs result in the exposure of CARD. Since a mutation at the ATP-binding site (Walker's A motif) was previously shown to inactivate these sensors, the ATPase activities of RIG-I and MDA5 may be involved in these conformational changes. In this step, K63-linked polyubiquitin on K172 and K788 by TRIM25 and Riplet, respectively, was found to promote and stabilize the oligomerization of RIG-I CARDS (Gack et al., 2007; Oshiumi et al., 2010).

The exposure of oligomerized CARDS leads to a signal relay to another CARD-containing protein, MAVS (also termed IPS-1, VISA, or Cardif; Figure 1) via CARD–CARD interactions (Meylan et al., 2005; Kawai et al., 2005; Xu et al., 2005; Seth et al., 2005). MAVS is localized on the mitochondrial outer membrane; therefore, RLR signaling from the cytosol is transmitted to an insoluble compartment in the cytoplasm. Recent *in vitro* reconstitution studies revealed that MDA5 formed a filament-like complex (also RIG-I-oligomer formation) on its ligand dsRNA (Peisley et al., 2013, 2011; Patel et al., 2013; Berke et al., 2012), which promoted the prion-like aggregation of MAVS (Hou et al., 2011). The artificial oligomerization of RIG-I using a chemical cross-linker in the cytoplasm activated the antiviral response in the absence of viral infection (Takamatsu et al., 2013). ATP hydrolysis induced filament disassembly, suggesting the transient formation of these filaments under physiological conditions. Mitochondrial fusion and fission are previously shown to be critical for the aggregate formation of MAVS (Onoguchi et al., 2010). In addition to mitochondria, several groups reported the importance of virus-induced stress granules (SGs) as critical loci for the activation of RLR (Onomoto et al., 2012). In several viral infections, viral dsRNA was found to activate PKR, leading to the phosphorylation of eIF2 α , which ultimately induced the formation of SGs, containing SG components, RLRs, and viral RNAs, to promote the activation of RLR (Onomoto et al., 2012; Yoo et al., 2014; Narita et al., 2014).

Signaling molecules including ubiquitin ligases (TRAFs) and kinase complexes (TBK1/IKK- α and IKK- α / β / γ) are recruited after the formation of MAVS aggregates and eventually activate the transcription factors IRF-3/7 and NF- κ B (Sato et al., 2000), leading to the production of type I IFNs and inflammatory cytokines, such as IL-6. In order to achieve this recruitment of IRF-3 and phosphorylation by TBK1 and IKK- α , recent studies revealed the importance of the phosphorylation of MAVS serine-rich clusters containing Ser442 (Liu et al., 2015). In certain cell types, RIG-I induces the production of IL-1 β through IPS-1/CARD9/NF- κ B

signaling upon viral infection (Poeck et al., 2010). Unlike MDA5, RIG-I is capable of triggering inflammatory responses by the direct formation of a signal complex with ASC and caspase-1 in an NLRP3-independent manner, suggesting a distinctive role of RIG-I in inflammatory responses (Poeck et al., 2010).

Various Viral Strategies to Block the RLR Signaling Pathway

To establish a successful infection, viruses must exploit various strategies to avoid recognition by RLRs and inhibit RLR signaling.

Hantaan virus and Crimean–Congo hemorrhagic fever virus modify the 5'ppp structure of their genomic RNA into 5'p to avoid being sensed by RIG-I (Habjan et al., 2008). The 5' end of picornavirus genomic RNA is linked to a viral protein, VPg (Vartapetian et al., 1980), which is considered to play a role in escaping detection by RIG-I.

RLRs and adapters of the signaling cascade are targeted by viruses. For example, the V proteins of several paramyxoviruses including Sendai virus, Newcastle disease virus, and parainfluenza virus 5 directly bind and antagonize MDA5 (Yoneyama et al., 2005; Rodriguez and Horvath, 2014, 2013). This interaction reportedly disrupts ATP hydrolysis and prevents the formation of MDA5 filamentation (Motz et al., 2013). In the case of measles and Nipah viruses, V proteins have been shown to repress MDA5 by targeting the phosphatases PP1 α / γ , thereby preventing the dephosphorylation of MDA5 CARDS (Davis et al., 2014). The N protein of respiratory syncytial virus (RSV) specifically binds to MDA5, leading to the relocalization of MDA5 to large inclusion bodies (Lifland et al., 2012). In addition, the NS1 and NS2 proteins of RSV were found to form a large and heterogeneous degradative complex on mitochondria, designated the NS-degradosome, which degraded RIG-I and several signaling molecules including TBK1, IRF3, and IRF7 (Goswami et al., 2013).

IAV NS1 is a well-known antagonist of RIG-I. The inhibitory properties of this NS1 protein have been partially attributed to its binding to dsRNA and RIG-I, resulting in the sequestration of RIG-I activation (Mibayashi et al., 2007). NS1 was also shown to directly associate with the ubiquitin E3 ligases TRIM25 and Riplet to block the ubiquitination of RIG-I CARDS and CTD, respectively (Rajsbaum et al., 2012). The leader protease of foot-and-mouth disease virus inactivates RIG-I by deubiquitination (Clementz et al., 2010; Wang et al., 2011). The M protein of SARS-CoV, Z protein of New World arenavirus, and glycoprotein G of human metapneumovirus were previously reported to bind to RIG-I, resulting in the sequestration of RIG-I in the cytoplasm from an essential signal adapter, MAVS (Siu et al., 2009; Fan et al., 2010; Bao et al., 2013). The NSs protein of severe fever with thrombocytopenia syndrome virus has recently been shown to interact with RIG-I, TBK1, and TRIM25 and induce the relocalization of these molecules into endosome-like structures for sequestration (Santiago et al., 2014).

MAVS is also a critical target for several viruses including HCV, which inactivates MAVS by the viral protease, NS3/4A (Meylan et al., 2005; Li et al., 2005). A recent study identified

Riplet as another target of HCV NS3/4A that subverted the activation of RIG-I, thereby favoring viral replication (Oshiumi et al., 2013). Enterovirus also reportedly cleaves MAVS and MDA5 by its protease designated 2Apro (Feng et al., 2014; Wang et al., 2013). The PB1-F2 protein of IAV was found to suppress MAVS-mediated signaling by directly binding to MAVS and decreasing the membrane potential of mitochondria (Yoshizumi et al., 2014; Varga et al., 2012). Measles virus has been shown to trigger the selective degradation of mitochondria by autophagy, termed mitophagy, which downregulated the expression of MAVS and attenuated RLR signaling (Xia et al., 2014).

In addition to the direct inhibition of molecules involved in signal pathways, viruses also counteract the production of IFN by repressing the formation of RLR-containing avSG. Poliovirus, EMCV, and Coxsackievirus type B3 disrupt avSG by cleaving Ras-Gap SH3 domain binding protein 1 (G3BP1) with the viral protease, 3C (Ng et al., 2013; Fung et al., 2013; White et al., 2007). A previous study reported that IAV NS1 inhibited the activation of PKR in addition to RIG-I, TRIM25, and Riplet in order to prevent the formation of avSG (Onomoto et al., 2012). Furthermore, several viruses, including Sendai virus, VV, human parainfluenza virus 1, and measles virus, were found to produce viral proteins that inhibit the formation of avSG by preventing the accumulation of dsRNA and activation of PKR (Onomoto et al., 2012).

RLR and Autoimmune Disorders

IFN plays a critical role in triggering immune responses against viruses. It has also been shown to assist in adaptive immune responses. For example, the expression of MHC class I on various cell types and costimulatory molecules on antigen-presenting cells is enhanced by IFN. IFN is also required for the promotion of T cell survival and its clonal expansion, B cell differentiation, and antibody production. Thus, defects in the production of IFN including an RLR deficiency markedly increase susceptibility to infecting viruses and decrease acquired immune responses, which can occasionally be fatal. On the other hand, recent studies revealed that the chronic or sustained activation of RLR-mediated signaling leads to autoimmunity.

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is characterized by various symptoms such as joint pain, a skin rash, and tiredness and is accompanied by elevated levels of type I IFN, termed the IFN signature. Nephritis, so-called lupus-nephritis, has been reported in severe cases. The central role of type I IFN has been suggested in the disease pathogenesis and treatments with anti-IFN α antibodies are known to ameliorate these symptoms. Several molecules including TLR7 and DNase I (Deane et al., 2007; Pisitkun et al., 2006; Napirei et al., 2000), the deficiency or overexpression of which is involved in the atypical production of IFN, were associated with the onset of SLE in a genome-wide association study (GWAS) and also in studies using mouse models.

GWAS also revealed that human SNPs in IFIH1 encoding human MDA5 such as rs1990760 (A946T) correlated with higher susceptibility to SLE (Robinson et al., 2011; Cunninghame Graham et al., 2011), suggesting that these SNPs cause the atypical activation of MDA5, thereby resulting in the aberrant production of IFN. Mice with the *Ifih1* missense mutation (G821S) were recently found to spontaneously develop lupus-like symptoms including nephritis and a skin rash (Funabiki et al., 2014). This is the first direct evidence to demonstrate that mutations in RLR were the direct cause of an autoimmune disease. In addition to the upregulation of type I IFNs and IFN-inducible genes, inflammatory cytokines including IL-6 and TNF were induced in multiple organs in this mutant mouse, reflecting ubiquitous expression of MDA5. The missense mutation, G821S enhanced the basal level of IFN by MDA5, but abrogated responsiveness to viral infections as well as the ATPase activity induced by dsRNA. The autoimmune phenotype was not observed in the mouse background of *Mavs*^{-/-}. These findings indicated that this mutation conferred constitutive activity on MDA5 rather than being hypersensitive to endogenous or viral RNA. Moreover, a simple increase in the dosage of the *Ifih1* gene did not induce spontaneous nephritis or any autoimmune symptoms (Crampton et al., 2012), suggesting that a dysregulation in MDA5 by a mutation may be essential for triggering autoimmunity. The further characterization of the mutant MDA5 will provide an insight into the precise mechanism responsible for the development of autoimmunity.

Aicardi-Goutieres Syndrome

Aicardi-Goutieres syndrome (AGS) is a rare inflammatory disease that particularly affects the brain and has the so-called IFN signature. There is no definitive cure to date, and the mechanistic basis underlying the onset of AGS has yet to be elucidated. A previous study reported that SNPs in the genes functioning in nucleic acid metabolism, including TREX1, SAMHD1, ADAR1, RNASEH2A, RNASEH2B, and RNASEH2C, were strongly linked to AGS (Crow and Rehwinkel, 2009). A deficiency in these genes may result in the accumulation of immune stimulatory RNA or DNA, leading to the chronic production of IFN.

Two groups recently identified seven mutations in IFIH1 in AGS patients by exome sequencing (Rice et al., 2014; Oda et al., 2014; Figure 4). Each of these mutations altered single amino acids within the helicase domain. Since patients had a single copy of the mutated allele, these mutations were suggested to be dominant. Different assays were adopted by the two groups; therefore, the activities of these MDA5 mutants remain controversial. Rice et al. (2014) used normal cultured cells to demonstrate that these MDA5 mutants exhibited hyperresponsiveness to ligand stimulation, which suggested the possible involvement of endogenous and/or viral RNA in the onset of AGS. Oda et al. used *Ifih1*^{-/-} cells to show that the mutant MDA5 was constitutively active without exogenous RNA. These findings suggested that these mutations led to a gain of function to facilitate IFN production in the absence of sensing endogenous RNA. Constitutive activity and hypersensitivity may depend on altered amino acids. An appropriate animal model

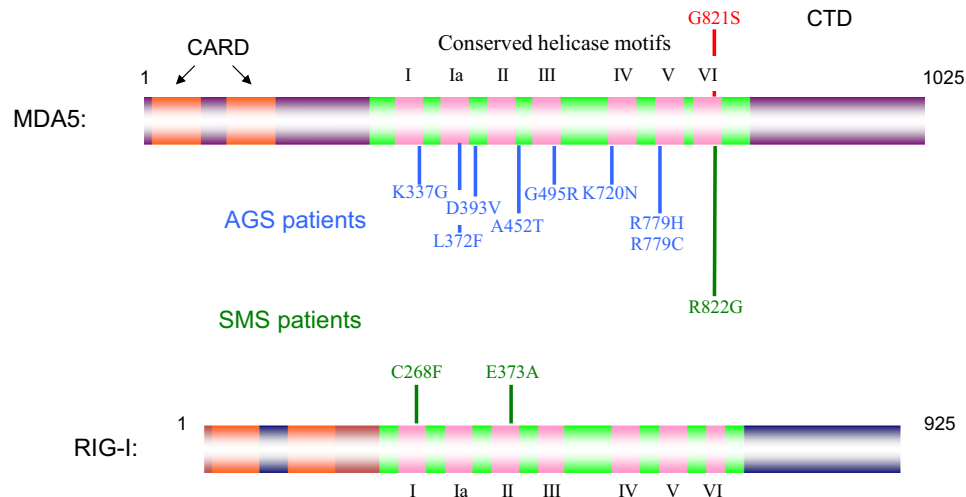


Figure 4 Mutations of RIG-I-like receptors (RLR) in autoimmune diseases. Mutations of RLR identified in Aicardi–Goutieres syndrome (AGS) and Singleton–Merten syndrome (SMS) patients are indicated in blue and green, respectively. G821S (red) is a missense mutation found in mice.

is awaited to further our understanding of this rare disease and develop appropriate treatments.

Singleton–Merten Syndrome

Singleton–Merten syndrome (SMS) is an extremely rare, multi-system disorder that is characterized by dental dysplasia, calcifications of the aorta, glaucoma, and osteoporosis. Jang et al. (2015) recently described a family affected by these symptoms and identified a missense mutation (E373A) in DDX58, encoding human RIG-I. RIG-I E373A leads to the constitutive production of type I IFN. Moreover, a further analysis of DDX58 in 100 individuals with congenital glaucoma resulted in the identification of another mutation (C268F). Glu373 and Cys268 of RIG-I reside in the ATP-binding motifs I and II in the Hel-1 domain, respectively (Figure 4). The constitutive activities and responsiveness to ligand RNA of these mutants have not yet been characterized. Although previous studies suggested a link between aberrant RIG-I expression and autoimmune diseases in the gut, evidence for the involvement of RIG-I remains scarce. Another group reported a novel missense mutation (R822G) in IFIH1 in SMS patients (Rutsch et al., 2015). MDA5 R822G exhibits constitutive activity. The position of this mutation is next to the G821S mutation in mice. R822 and G821 are conserved in humans and mice, with mutations in these residues resulting in a constitutively active phenotype.

The three autoimmune diseases discussed above exhibit common and unique phenotypes, suggesting that they may share a basic cause for the symptoms of the constitutive production of type I IFN. However, it currently remains unclear whether their unique phenotypes, which are very much diverged, are attributed to mutations in the IFIH1 gene.

Type 1 Diabetes and Others

Type 1 diabetes (T1D) is caused by a defect in insulin production from pancreatic cells by genetic and immune factors.

Viral infections and antiviral host responses have been suggested to trigger autoimmune responses against pancreatic β cells in the early stage of T1D. Nejentsev et al. (2009) reported that two canonical splice site variants in IFIH1, a nonsense variant and missense substitution, were protective against T1D. These loss-of-function mutations in IFIH1 may attenuate innate immune responses against viruses. Enteroviruses, especially coxsackievirus B4, have been strongly implicated in the triggering of T1D. Moreover, MDA5 acts as a sensor for detecting the Picornaviridae family, including coxsackievirus. Further studies on IFIH1 may provide us with a clear connection between coxsackievirus infection and the development of T1D (Jaidane and Hober, 2008). Previous studies examined GWASs, and associated IFIH1 SNPs with the risk of psoriasis, selective IgA deficiency, and dilated cardiomyopathy (Ferreira et al., 2010; Dou et al., 2014). An autoantibody to MDA5 was specifically detected in a subpopulation of patients with clinically amyopathic dermatomyositis (Sato et al., 2009; Nakashima et al., 2010). Although the relationship between anti-MDA5 and the disease phenotype is strong, the causative relationship currently remains unknown.

Future Perspectives

Since the discovery of RIG-I as a viral sensor, intensive studies during the last decade have definitively revealed the physiological significance of RLRs in triggering antiviral innate immune responses, especially against RNA viruses and also to some DNA viruses. In addition, our understanding of the interaction between hosts and viruses has been steadily advanced by elucidating sensing and signaling mechanisms by RLRs and the clever strategies of each virus to escape from host immunity. However, infectious diseases by various existing and emerging viruses have yet to be controlled. Moreover, inappropriate RLR signaling has been strongly linked to the development of autoimmune diseases. Thus, in

addition to basic research, clinical and translational studies of RLR will be of importance in the near future.

See also: Cytokines and Their Receptors: Inflammasomes; Interferon α/β . Molecular Aspects of Innate Immunity: The NOD-Like Receptors. Signal Transduction: Adapter Molecules in Immune Receptor Signaling.

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