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Function and Regulation of Retinoic Acid-Inducible Gene-I

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Abstract

Antiviral innate immunity is triggered by sensing viral nucleic acids. RIG-I (retinoic acid-inducible gene-I) is an intracellular molecule that responds to viral nucleic acids and activates downstream signaling, resulting in the induction of members of the type I interferon (IFN) family, which are regarded among the most important effectors of the innate immune system. Although RIG-I is expressed ubiquitously in the cytoplasm, its levels are subject to transcriptional and post-transcriptional regulation. RIG-I belongs to the IFN-stimulated gene (ISG) family, but certain cells regulate its expression through IFN-independent mechanisms. Several lines of evidence indicate that deregulated RIG-I signaling is associated with autoimmune disorders. Further studies suggest that RIG-I has functions in addition to those directly related to its role in RNA sensing and host defense. We have much to learn and discover regarding this interesting cytoplasmic sensor so that we can capitalize on its properties for the treatment of viral infections, immune disorders, cancer, and perhaps other conditions.

Keywords

retinoic acid-inducible gene-I; innate immunity; RNA virus

I. Introduction

Vertebrate animals have developed innate and adaptive immune systems that defend them against microbial and other infections.[1-3] The innate immune system is the first line of cellular defense against foreign invasion. When pathogens enter the cytoplasm, they are recognized by germline-encoded pattern-recognition receptors (PRRs) that initiate signaling events, resulting in the production of cytokines such as type I interferons (IFNs).[2,3] IFNs have antiviral, antiproliferative, and immunomodulatory activities, and thus play crucial roles in host defense.[4] The elucidation of mechanisms that link microbial invasion, engagement of PRRs, and activation of IFN responses has been an area of active investigation in recent years. The Toll-like receptor (TLR) family comprises a sub-group of highly conserved PRRs.[5] Thus far, 10 isoforms (TLR1–TLR10) have been identified in humans, and mice are known to express an additional variant (TLR11). TLRs are roughly divided into two groups based on their subcellular localization. Cell surface-expressed TLRs (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR11) recognize viral proteins and bacterial and fungal cell wall components. Other members of the family (TLR3, TLR7, TLR8, and TLR9)

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are expressed in intracellular compartments such as endosomes and sense mainly nucleic acids.[5,6] Specifically, TLR3 senses double-stranded RNA (dsRNA) and TLR7 and TLR8 detect single-stranded RNA (ssRNA) of viral origin. However, TLR3-null cells respond to synthetic dsRNA, suggesting that additional mechanisms participate in the recognition of viral RNA.[7]

Two additional families have recently been characterized as members of the PRR superfamily: nucleotide-oligomerization domain (NOD)-like receptors (NLRs) and RIG-I (retinoic acid-inducible gene-I)-like receptors (RLRs). NLRs sense bacterial peptidoglycans in the cytoplasm, and RLRs detect viral nucleic acids in the cytosol.[8] Three members of the RLR subfamily have been characterized thus far, including RIG-I, MDA5 (melanoma differentiation-associated gene 5), and LGP2 (laboratory of genetics and physiology 2). Each member senses specific viral RNA structures; the fact that RIG-I recognizes viruses that broadly impact human health, such as influenza and hepatitis C viruses, has sparked the interest of virologists and immunologists across the world.

This review is centered on structural and functional aspects of RIG-I. First, we discuss RNA features required for recognition by RLRs. Second, we provide current views regarding the role played by individual RIG-I domains in auto-repression, RNA binding, and signal transduction. Third, we present key studies that led to the elucidation of signaling events that follow RIG-I activation. Finally, we discuss viral- and host-mediated mechanisms that control the function and expression of RIG-I. Not surprisingly, deregulation of RIG-I-mediated events has been reported to contribute to the pathogenesis of human diseases, with an emphasis on autoimmune disorders. The final section of this review briefly discusses key work addressing this important issue.

II. Retinoic Acid-Inducible Gene-I-like Receptors

A. Family of RNA Sensors

RLRs (RIG-I, MDA5, and LGP2) are members of the DExH/D (Asp-Glu-X-His/Asp)-box RNA helicase family that function as cytoplasmic RNA sensors.[9] These proteins participate in a wide range of biological processes, including transcription, translation, mRNA splicing, ribosome biogenesis, and RNA decay.[7] DExH/D-box proteins harbor four highly conserved motifs and utilize hydrolysis of NTPs to NDPs as an energy source.[10,11] RLRs are expressed in most cell types and are essential for the eradication of viruses through the innate antiviral activity of IFNs.[9]

B. Initial Studies

1. Retinoic Acid-Inducible Gene -I—In 1997, a group from the Shanghai Institute of Hematology cloned novel *all-trans* retinoic acid (ATRA)-inducible genes from leukemia cells and named them “RIG-A, RIG-B, RIG-C,” and so on. The nucleotide sequences were submitted to GenBank®, and RIG-I was identified as one of the genes whose expression was induced by ATRA treatment.[12] Following the discovery of RIG-I, it was reported that stimulation of vascular cells with lipopolysaccharide (LPS), a well-recognized inducer of inflammation, led to up-regulation of RIG-I expression.[13] Additional work showed that a variety of cells respond to IFN-γ stimulation by up-regulating RIG-I levels,[14-17] thus supporting a role for the cytoplasmic sensor as a mediator of inflammation and innate immunity.

In 2002, Tabara et al. characterized features of the RNA-induced silencing complex, a protein complex that plays a key role in gene silencing in *Caenorhabditis elegans*. Mass-spectrometric studies revealed that one of the components of this complex was a helicase called Dicer-related helicase (DRH), which is required for dsRNA-mediated post-

transcriptional gene silencing.[18] The helicase domain of DRH is highly homologous to that of RLRs, suggesting evolutionary links between RNA interference and the RLR system with regard to the detection of foreign RNA.[19]

2. Melanoma Differentiation-Associated Gene 5—The discovery of MDA5, like that of RIG-I, was based on studies aimed at the identification of novel proteins involved in defined biological processes. Investigators focused on the characterization of proapoptotic signaling pathways and searched public databases to identify novel proteins harboring CARD (caspase recruitment domain) motifs.[20] These studies led to the discovery of a CARD-containing mouse sequence that turned out to be Mda5.[20] In addition, Kang et al. identified human MDA5 (also known as Helicard) as having dsRNA-dependent ATPase activity with melanoma growth-suppressive properties.[21]

3. Laboratory of Genetics and Physiology 2—The discovery of LGP2 was based on studies from a research team interested in mammary gland development and remodeling.[22] These processes are regulated by two members of the STAT (signal transducer and activator of transcription) family, STAT3 and STAT5. The close proximity of *Stat3*, *Stat5a*, and *Stat5b* in a region of mouse chromosome 11[23] led Cui et al. to hypothesize that this locus might harbor additional genes involved in mammary gland development.[24] This approach led to the discovery of *Lgp1* and *Lgp2*, two novel genes highly expressed in normal mammary tissue and mammary tumors. LGP2 was found to be a cytoplasmic protein harboring a DExH/D-box motif and a carboxy-terminal helicase domain.[24]

C. Comparative Analyses of Structural RLR Features

Structural examination of the three RLRs provided important clues related to the role played by individual domains in viral recognition, regulation of activity, and signal transduction. All RLRs harbor at least two functional domains: a central DExD/H-box helicase domain and a carboxy-terminal repressor domain (Fig. 1).[25,26] Two amino-terminal CARDs are components of RIG-I and MDA5, but not LGP2 (Fig. 1). Overexpression of CARDs induces constitutive signaling independently of viral infection,[9,27] demonstrating that these domains are required for the transduction of signals following activation of RIG-I and MDA5.[26] The mechanism involves homotypic interaction with a CARD-containing adaptor molecule, MAVS (mitochondrial antiviral signaling),[28] also known as IPS-1 (IFN-β promoter stimulator-1),[29] VISA (virus-induced signaling adaptor),[30] and Cardif (CARD adaptor-inducing interferon-β).[31] Only the terminal CARD of RIG-I and MDA5 interacts physically with MAVS, but both CARDs are required for signaling.[32] Constructs lacking these signatures have dominant negative effects.[33] Conformational issues and interactions between CARDs and the carboxy-terminal domain of RLRs regulate homotypic interaction with MAVS, and thus modulate signal transduction (see below). CARDs negatively regulate the ATPase activity of RIG-I in the absence of dsRNA; this mechanism may constitute a safeguard to prevent signaling in the absence of viral RNA stimulation.[27]

The helicase/translocase domain expressed by members of the RLR family was named owing to its characteristic structural signature (Fig. 1).[34] Confirmation that RIG-I encodes a functional helicase was recently provided by Marques et al., who showed that full-length RIG-I exhibits complete unwinding activity toward dsRNA harboring a 3′ overhang longer than 15 nucleotides.[35] ATP hydrolysis is necessary but not sufficient for unwinding in vitro.[36-38] It is not clear, however, whether RIG-I unwinds dsRNA duplexes or simply translocates on the RNA molecule in vivo.[32] In fact, it has been proposed that the helicase domain induces an ATP-dependent conformational change that facilitates signaling through CARDs.[39] In addition, the helicase domain appears to bind distinct RNA motifs and cooperate with the carboxy-terminal domain to optimize the affinity of the interaction.[38,40]

The helicase domains of MDA5 and RIG-I are only about 35% identical, suggesting that these domains participate in the determination of substrate specificity.[41] The *primary* RNA-binding site, however, is located in a different region of RIG-I (see below).[38] In summary, the central helicase domain seems to utilize conformational changes to bridge RNA-binding events occurring at the carboxy-terminal end to the amino-terminal end, thus facilitating transduction of signals through the CARDs of RIG-I.

RIG-I and LGP2, but not MDA5, harbor a carboxy-terminal repressor domain that has been proposed to exert inhibitory functions by interacting with other protein regions such as the CARD and helicase domains (Fig. 1).[33] In the absence of viral infection, the repressor domain represses RIG-I activity owing to its ability to fold in such a manner that CARDs are shielded from interaction with MAVS.[32] A surface enriched with acidic patches has been associated with these repressor domain-regulatory functions.[38] During viral infection, RIG-I activation and signaling occur through conformational changes that stimulate self-association and allow CARD/MAVS interaction.[33,42,43]

A stretch of amino acids comprised of carboxy-terminal domain residues 792 to 925 that overlap with the repressor domain has been demonstrated to be the primary RNA-binding domain.[38] Structural analyses using X-ray crystallographic and nuclear magnetic resonance approaches revealed that this region harbors a positively charged groove that may be the RNA-binding site; in addition, a zinc-binding domain seems to be required for optimal interaction.[38,44] Interestingly, the groove is different in LGP2 and MDA5, raising the possibility that this domain may account for the well-established ability of RIG-I and MDA5 to recognize different types of RNA viruses.[44,45] Additional insights into the role of the carboxy-terminal domain were provided by recent studies in *lgp2*$^{-/-}$ mice.[25] LGP2, a CARD-less RLR unable to transduce activation signals, has been proposed to negatively regulate RIG-I-mediated signaling through a variety of mechanisms, including sequestration of dsRNA, inhibition of RIG-I interaction with MAVS, and blockade of MAVS signaling downstream of RIG-I.[33,46-49] However, Satoh et al. have shown that, with the exception of influenza virus, LGP2 acts as a *positive* regulator of viral recognition mediated by MDA5 and RIG-I.[25] Their results suggest that LGP2 is involved in primary sensing of RNA viruses upstream of MDA5 and RIG-I. The authors speculate that LGP2 facilitates recognition of dsRNA by RIG-I and MDA5, either by removing proteins from viral ribonucleoprotein complexes or by unwinding complex RNA structures.[25] These potential effects may require the carboxy-terminal domain of LGP2, as this region was shown to be responsible for binding to the termini of dsRNAs.[38,44,50] It is possible that LGP2 has both positive and negative functions and that the type of response elicited is defined by the structure of the infecting virus.[19,49] In summary, the carboxy-terminal end of RLRs is the primary site for RNA binding and it modulates RLR activity. These effects require conformations that either repress function under basal conditions or induce oligomerization following stimulation with RNA.

D. Ligand Recognition

Two recent reviews highlight important structural features required for viral sensing; readers are encouraged to consult these excellent articles for further details.[32,51] Accumulating evidence indicates that RLRs can distinguish among different RNA ligands; this is evidenced when one compares structural features of MDA5 and RIG-I substrates. RIG-I has been reported to recognize RNA derived from 25 viruses (Table 1).[9,46,52-65] It has specificity for RNA viruses and does not bind viral dsDNA (except Epstein-Barr virus, Table 1). Until recently, it was thought that RIG-I recognized only dsRNA, and it was assumed that ssRNA viruses were sensed when they replicate, a process during which dsRNA is produced. However, Habjan et al. recently showed that ss(−)RNA does not generate substantial amounts of dsRNA, suggesting that this mechanism may not account for

the ability of RIG-I to recognize ssRNA viruses.[55] This issue was resolved when two groups demonstrated that ssRNA harboring a 5′-triphosphate (5′ppp) group activates RIG-I-mediated type I IFN induction.[62,66] Consistently, deficiency of RIG-I disrupted responses to ssRNA viruses that harbored this structural signature, such as Sendai virus, Newcastle disease virus, and hepatitis C virus.[45] In contrast, ssRNA viruses harboring a *single* phosphate group at the 5′-end of their genomes (i.e., members of the Bornaviridae family) escape recognition by RIG-I.[55,67] Most RNA viruses contain 5′ppp in their genomes and antigenomes, but cellular RNAs lack exposed 5′ppp as a result of mRNA capping or processing of 5′ppp into monophosphates.[68] However, two groups recently showed that pure ssRNA with a 5′ppp group is *unable* to activate RIG-I, and demonstrated that the optimal agonist is blunt-ended 5′ppp dsRNA at least 20 base pairs in length (Fig. 2).[36,37] The investigators proposed that a “panhandle” structure generated by base-pairing of 5′ and 3′ regions of the viral genome is recognized by RIG-I.[36,37] This model provides a mechanism to explain how RIG-I detects negative-strand RNA viruses that lack long dsRNA but contain short double-stranded 5′ppp RNA in certain regions of their single-stranded genomes.[36,37] Marq et al. contributed the observation that if the 5′ppp group is unpaired (as in members of the Arenavirus family), recognition by RIG-I is prevented.[69]

A second factor that affects the suitability of RNAs as substrates for RLRs is the length of these molecules. For example, polyinosinic-polycytidylic acid, also called poly (I:C), a synthetic dsRNA, is thought to activate IFN responses following recognition by MDA5.[41,45] However, when the length of poly (I:C) is decreased, it becomes a ligand for RIG-I (Fig. 2).[70] This feature likely represents in vivo substrate preferences, as RIG-I and MDA5 are activated by viral dsRNAs in a length-dependent fashion.[70]

Structural features unique to certain families of RNA viruses may also affect recognition by RLRs. For example, encephalomyocarditis virus, a member of the Picornaviridae family, is a unique RNA virus that activates type I IFN induction exclusively through MDA5.[41] It was suggested that structural characteristics unique to encephalomyocarditis virus, such as the presence of a polyC acid tract in the 5′-untranslated region (UTR), may define its suitability as an MDA5 substrate.[41] Related studies have shown that, in addition to the 5′ppp, RIG-I recognizes the 3′-UTR of some viruses such as hepatitis C virus. Within this domain, a homopolymeric poly(U/UC) region unlikely to form stable secondary structures was mapped as that responsible for activation of IFN responses.[71] Deletion of the 3′-UTR was shown to elicit decreased signaling, a result consistent with a critical role for this region in RIG-I-mediated innate immune responses.[72] In summary, RIG-I recognizes the presence of 5′ppp groups in viral genomes and somewhat short, blunt-ended dsRNAs. Accordingly, dsRNA generated by infection of certain DNA viruses is also recognized by RIG-I. The elucidation of RNA sequences and structural features defining recognition by RLRs is likely to significantly affect our ability to capitalize on these properties to limit viral infection of humans, animals, and plants.

E. Signal Transduction Domains

Our understanding of the mechanisms through which RIG-I transduces signals following viral stimulation has been significantly advanced by two breakthrough discoveries. Pioneering work by Yoneyama et al. established that RIG-I has antiviral properties and plays a key role in dsRNA-mediated IFN-β expression.[9] The IFN-β gene harbors one of the best-characterized higher eukaryotic enhancers.[73] Gene expression requires coordinate activation and DNA binding of the activating transcription factor-2/c-Jun, interferon regulatory factor (IRF) 3, IRF7, and nuclear factor (NF)-κB (p50/p65).[73] Upon viral infection, these transcription factors are recruited gradually on a specific enhancer sequence to form the “enhanceosome.”[73] In the presence of cytoplasmic dsRNA, RIG-I activates NF-κB and IRF3. As noted above, CARDs are required for activation, and mutants in this region

fail to transduce signals. Each CARD seems to have specificity for individual downstream targets.[9]

A second major breakthrough in this field was the discovery by four independent groups of MAVS,[28,29,30,31] a CARD-containing adaptor molecule that, as previously mentioned (section IIC), links RIG-I and IFN-β signaling. MAVS was originally identified from large-scale screening of human genes that activate the NF-κB and MAPK signaling pathways.[74] MAVS consists of an amino-terminal CARD, a proline-rich region near the amino-terminus, and a carboxy-terminal transmembrane domain. X-ray crystallographic studies revealed that the CARD in MAVS adopts the classic CARD fold and is homologous to the first CARD of RIG-I.[75] The transmembrane domain allows MAVS to localize to the outer mitochondrial membrane, suggesting that the mitochondria are a platform for MAVS-mediated signaling.[28] Indeed, the function of MAVS is impaired when the transmembrane domain is deleted or replaced; restoring mitochondrial localization by expression of the membrane targeting domain of Bcl-2 or Bcl-xL also restores function.[28] Interaction between CARDs from RIG-I and MAVS results in the activation of type I IFNs,[28-31] a process during which the signal originating in RIG-I is transferred to MAVS.

F. Modulation of RIG-I Activity Through Ubiquitin-Dependent and Related Mechanisms

Recent studies have led to the identification and characterization of host proteins that regulate the activity of RIG-I, and thus affect its ability to transduce signals following viral stimulation. Derivatization of proteins with lysine 63-linked ubiquitin chains is a post-translational modification known to regulate intracellular signaling.[76] TRIM25, a member of the tripartite motif (TRIM) protein family,[77] is an E3 ubiquitin and IFN-stimulated gene 15 (ISG15) ligase reported to bind to the amino-terminal CARD of RIG-I and conjugate lysine 172 located in the internal CARD to a lysine 63-linked ubiquitin chain (Fig. 3).[32,78] Inhibition of this step by mutation or deletion of lysine 172 prevents interaction of RIG-I and MAVS, and thus inhibits IFN production.[79] Cells lacking TRIM25 have impaired production of type I IFNs in response to viral infection. Interestingly, TRIM25 is inhibited by NS1, an influenza protein known for its ability to antagonize the immune system.[80] NS1 directly interacts with TRIM25 and prevents activation of RIG-I,[81] emphasizing the importance of TRIM25 in RIG-I function.

While it is clear that lysine 63 ubiquitin chains play an important role as RIG-I activators, a recent study by Zeng et al. challenged whether *covalent derivatization* of RIG-I is necessary for activation. This group developed a cell-free system consisting of RIG-I, mitochondria, cytosol, RNA, and ubiquitination enzymes (including TRIM25) that was used to mimic viral infection in intact cells.[82] The studies showed that RNA promotes recruitment of lysine 63-linked ubiquitin chains to the CARDs of RIG-I and that *unanchored* ubiquitin chains potently activate RIG-I and function as endogenous RIG-I ligands in human cells.[83] These results suggest that while the association between lysine 63-linked ubiquitin chains and CARDs of RIG-I is essential for activation, RIG-I ubiquitination per se may be dispensable.[82] Recently, RNF135/Riplet/REUL was reported as another E3 ligase that can positively regulate RIG-I signaling (Fig. 3).[84,85] The actions of RNF135 are centered on different domains of RIG-I, as this protein interacts with the repressor domain and with the helicase domain but not with CARD and catalyzes lysine 63-linked polyubiquitination of the carboxy-terminal region of RIG-I. It is not clear, however, how this modification regulates downstream signaling.

The cellular need to carefully monitor the activity of RIG-I and other RLRs likely resulted in the evolution of compensatory strategies to regulate ubiquitin-mediated activation of these sensors. The tumor suppressor CYLD, a deubiquitinating enzyme, associates with the CARD domain of RIG-I and removes lysine 63-linked ubiquitin conjugated by TRIM25

(Fig. 3).[86] Thus, CYLD may provide a natural mechanism to limit RIG-I-mediated antiviral signaling.[87] An additional strategy to limit activation through this pathway was recently proposed by Nistal-Villán et al.[88] These investigators showed that phosphorylation of serine 8 located within the first CARD of RIG-I inhibits its ability to interact with TRIM25, and thus blunts IFN-β production (Fig. 3). This group also reported that phosphorylation of threonine 170 in the second CARD domain of RIG-I has a negative impact on RIG-I ubiquitination and activation.[89] Derivatization of RIG-I with ubiquitin has also been shown to act as a negative regulator of RIG-I. A study using a yeast two-hybrid screen identified RNF125, a ubiquitin E3 ligase, as a protein that specifically interacts with the CARD and helicase domains of RIG-I (Fig. 3).[86] RNF125 efficiently conjugates ubiquitin to the CARDs of RIG-I, and this modification leads to enhanced degradation of RIG-I through the proteasome.[90] We found that this process can itself be inhibited under some circumstances. Our studies have shown that HSP90 (heat shock protein 90) interacts with RIG-I in a CARD-independent fashion and that disrupting this association prevents downstream signaling.[91] Studies using HSP90 inhibitors suggested that the role of HSP90 in this setting is to protect RIG-I from ubiquitin-mediated proteasomal degradation. An additional mechanism to negatively regulate RIG-I involves modification with a ubiquitin-like protein known as ISG15 (Fig. 3).[92,93] Overexpression of ISG15 combined with specific enzymes of the ubiquitination system decreases or delays basal and stimulated IFN-β promoter activity. Several ISG15-derivatized RIG-I species can be detected following ISG15 overexpression, but it is not clear whether these represent multiple sites of derivatization or if poly-ISG15 links to RIG-I at a single site. The mechanism whereby ISGylation dampens RIG-I signaling likely involves degradation of RIG-I through a proteasome-independent pathway.[94] In summary, it is apparent that RIG-I is in a constant state of flux between free forms and species derivatized or associated with ubiquitin, ISG15, and possibly other proteins. The balance between these forms is likely dictated by multiple contributions that ensure optimal signaling when the need arises, and that prevent unnecessary responses under normal conditions.

III. Signaling Events Mediated by RIG-I

A. The MAVS Pathway

Following recognition of RNA by RIG-I, a conformational change allows interaction between the CARDs of RIG-I and MAVS.[42,43] This process is independent of the helicase activity of RIG-I but requires its translocase activity and ATP.[9,38,95] The assembly of RIG-I/MAVS has been thought to take place exclusively on mitochondria, but recent work by Dixit et al. has shown that MAVS is also found on peroxisomes.[96] The data suggest that peroxisomal MAVS may establish an early and transient response to halt or delay viral replication, followed by robust and prolonged signaling driven by the mitochondrial MAVS pathway.[97] A trans-membrane protein known as STING (stimulator of interferon genes) resides in the endoplasmic reticulum and the mitochondrial membrane, directly interacts with MAVS, and has been reported to be a critical scaffold for protein assembly.[98,99] In addition, recent studies have shown that the DEAD (Asp-Glu-Ala-Asp)-box helicase DDX3 (DEAD box polypeptide 3) is a component of the MAVS complex owing to its ability to constitutively associate with MAVS through its carboxy-terminal end.[100,101] DDX3 is proposed to serve as an initial sensor of RNA in unstimulated cells, and may intensify signaling from MAVS when the levels of RIG-I are low.[100] In addition to the ubiquitin-mediated and related strategies (described in section IIF), naturally occurring mechanisms have evolved to inhibit interaction between MAVS and RIG-I, presumably to prevent unwanted or prolonged activation of antiviral signaling that may be harmful for host survival.[99] An Atg5-Atg12 conjugate, a regulator of autophagy, has been shown to inhibit interactions between MAVS and RIG-I.[102] NLRX1, a protein expressed on the outer mitochondrial membrane, is a negative regulator of RLR-mediated antiviral signal owing to

its ability to directly interact with the CARD in MAVS.[103] Finally, gC1qR, a C1q receptor implicated in the regulation of innate and adaptive immunity, has been shown to translocate to the mitochondria, interact with MAVS, inhibit the formation of RIG-I/MAVS complexes, and dampen antiviral signaling.[104]

The formation of the MAVS complex is followed by engagement of at least three signaling axes involved in the activation of IRFs, NF-κB, and apoptosis.[19,105] We will discuss each of these pathways separately, with the understanding that significant overlap exists among them. This section is limited to a discussion of RIG-I-mediated events, but it is important to emphasize that the MAVS pathway can be engaged by a variety of receptors that detect viral nucleic acids.

B. TRAF-Mediated IRF Activation Through Non-Canonical IKKs

A number of tumor necrosis factor (TNF) receptor-associated factor (TRAF) family members have been shown to participate in signaling events downstream of RIG-I/MAVS complex formation (Fig. 4).[99] TRAF3, an E3 ligase for lysine 63-linked polyubiquitination, directly interacts with the TIM (TRAF-interacting motif) in MAVS.[106,107] TRAF3-dependent addition of K63-linked ubiquitin chains to components of the MAVS signaling complex is crucial for the activation of IFN production.[108] In addition, auto-ubiquitination of TRAF3 enhances its binding to NF-κB essential modulator (NEMO, also known as IKKγ), a canonical I-κb kinase (IKK) that regulates the IKK complex, leading to activation of IRF3.[109] Consistently, selective cleavage of lysine-63-linked polyubiquitin chains on TRAF3 through deubiquitinating enzyme A (DUBA) blunts IFN responses.[110] During later phases of viral infection, the E3 ligase Triad3A adds lysine 48-linked polyubiquitin chains to TRAF3 and targets it for degradation through the proteasome.[111] These observations suggest dual, temporal regulation of TRAF3 through derivatization with different types of ubiquitin polymers. It was recently reported that TRAF5 also plays an important role in IRF3/7 activation downstream of MAVS (Fig. 4).[112]

NEMO has been shown to physically interact with TANK (TRAF family member-associated NF-κB activator) to mediate recruitment of IKKε and TBK1 (TANK-binding kinase 1) to the complex (Fig. 4).[113,114] A NEMO mutant lacking its TANK-binding domain failed to recruit IKKε to the RIG-I/MAVS complex, thus preventing IFN production induced by viral infection.[113] In the absence of viral stimulation, SIKE (suppressor of IKKε) forms a complex with TBK1 and/or IKKε that prevents these kinases from interacting with RIG-I or IRF3.[115]

The transmission of activation signals to the non-canonical IKKs TBK1 and IKKε results in phosphorylation and dimerization of IRF3 and IRF7.[107,116] This cascade leads to IFN-α production (Fig. 4) and contributes to IFN-β synthesis. IFN secretion from infected cells initiates autocrine-and paracrine-signaling cascades through type I IFN receptor that result in up-regulation of more than 100 different genes and the establishment of an antiviral state in both infected and neighboring uninfected cells.[32]

Additional regulatory components of this signaling axis include NAP1 (NAK-associated protein 1), initially characterized as an activator of IKK-related kinases, and DDX3 (see above).[117] NAP1 interacts with IKKε and TBK1,[118] and also physically interacts with MAVS. The coiled-coil motif of NAP1 is highly homologous to those of NEMO and TANK, suggesting that the three proteins utilize these domains for kinase binding and subsequent regulation of downstream signaling.[118,119] The precise nature of the interaction(s) between NAP1 and other members of the complex remains to be established. The aminoterminal end of DDX3 associates with IKKε and exerts positive effects as a part of the TBK1/IKKε complex that activates IRF3.[117]

C. RIG-I/MAVS Signaling Through the Canonical IKK Complex

In addition to TRAF3/5, TRAF2 and TRAF6 have been reported to associate with MAVS and transmit signals to IKKs.[30] Unlike TRAF3/5, which recruit canonical and non-canonical IKK complexes, TRAF2/6 are primarily involved in canonical signaling leading to NF-κB activation.[30,107] The relay of signals through this axis involves participation of several proteins that harbor an intercellular death domain present in multiple proteins that trigger apoptosis (Fig. 5). These include FADD (Fas-associated death domain) and RIP1 (receptor interacting protein 1).[29,120] Deficiency of these proteins prevents activation of IFN or NF-κB signaling in response to dsRNA treatment.[120,121] FADD and RIP1 interact with the carboxy-terminal portion of MAVS, suggesting possible connections between MAVS signaling and death domain-expressing proteins during innate immune responses. In addition, TRADD (TNF receptor-associated death domain), a crucial adaptor of TNF receptor I, was also demonstrated to be essential for RIG-I-MAVS-dependent signaling.[122] According to Michallet et al., a TRADD-containing complex that includes RIP1 and FADD forms a central molecular signaling unit that assembles on the intermediary region of MAVS following activation of RIG-I by viral nucleic acids.[122] An additional step necessary for NF-κB activation is FADD-mediated engagement of caspase-8 and/or caspase-10. It is well established that these caspases are cleaved and become activated following dsRNA exposure, but the precise details that link caspase activation to the NF-κB pathway remain to be established.[121] TRAFs recruited by MAVS induce RIP1 ubiquitination and association of NEMO, a key regulator of IKKs. The canonical IKK complex consisting of IKKα, IKKβ, and NEMO phosphorylates IκB, followed by its proteasomal degradation, translocation of NF-κB to the nucleus, and activation of proinflammatory cytokines and other target genes (Fig. 5).[123]

D. RIG-I/MAVS Induces Two Effector Arms That Converge on NEMO

Sections IIIB and IIIC summarize evidence supporting RIG-I/MAVS-mediated activation of two distinct pathways: the IRF3/7 and the canonical NF-κB pathways (Figs. 4 and 5, respectively). A common feature between these axes is a requirement for NEMO as an adaptor protein that organizes the assembly of IKKs into activated high-molecular-weight complexes.[124] In the NF-κB pathway, NEMO is required for recruitment of IKKα and IKKβ to RIG-I/MAVS, activation of NF-κB, and induction of inflammatory cytokine expression. In contrast, the IRF3 pathway requires interaction between NEMO and the TBK1 adapter TANK for efficient coupling of the RIG-I/MAVS complex to IRF3 activation.[113] These considerations indicate that NEMO is the final component shared between the NF-κB and the IRF3 signaling pathways engaged following RIG-I/MAVS activation.[125] Formal evidence supporting this paradigm was elegantly presented by Liu et al.[125] These investigators used an alternatively spliced form of NEMO lacking an amino-terminal domain. The splice variant was found to efficiently mediate cytokine-induced, canonical NF-κB activation but failed to couple RIG-I/MAVS signaling to the TANK-IKKε complex, thus preventing IRF3 activation. This inability to uncouple IRF3- and NF-κB-dependent effects convincingly showed the existence of different mechanisms for NEMO-mediated regulation of canonical and non-canonical IKK signaling following RIG-I/MAVS activation. While both IRF3 and NF-κB signaling are important responses to viral infection, they serve different purposes: IRF3 plays a role in mucosal antiviral responses[113] and NF-κB participates in the initiation of mucosal inflammation and in adaptive immunity.[125] Appropriate regulation and temporal coordination of these two signaling arms downstream of NEMO likely plays an important role in the resolution of viral infection.

E. IRF3 Plays a Role as a Mediator of dsR-NA-Induced Apoptosis

In addition to transcription factor-driven up-regulation of IFN genes and ISGs, both of which are essential for innate and adaptive antiviral defenses, cells utilize apoptosis to limit

viral spreading at the cost of cellular death. In recent elegant studies, Chattopadhyay et al. found that IRF3 has pro-apoptotic functions in addition to its role as a transcriptional activator (Fig. 6).[126] Engagement of RIG-I was shown to activate the apoptotic activity of IRF3 through a distinct signaling pathway that required the participation of several proteins. The investigators clearly showed that RIG-I, MAVS, TRAF3, and TBK1, all of which are necessary for gene induction (Fig. 4), are also required for the apoptotic effects of IRF3 (Fig. 6). In addition, the adaptor proteins TRAF2 and TRAF6 were shown to be required for IRF3-mediated apoptosis but not for IRF3-mediated induction of gene expression (Fig. 6).[126] Mechanistically, a BH3 domain located in the carboxy-terminal region of IRF3 was shown to enable interaction with pro-apoptotic Bax; the IRF3/Bax complex is subsequently translocated to the mitochondria, where activation of the intrinsic apoptotic pathway takes place (Fig. 6). Future studies should be centered on elucidating how TRAF2 and TRAF6 contribute to IRF3-dependent apoptotic responses.[127]

IV. Evasion of RIG-I-Mediated Antiviral Responses

Even though the innate immune system has developed sophisticated mechanisms for viral eradication, infections still occur.[128] Viruses are skilled pathogens that have developed a number of survival mechanisms to effectively evade detection. First, structural features of viral RNAs and virally encoded proteins prevent recognition by RIG-I and allow certain viruses to escape detection. Second, some viruses produce proteins that inhibit key steps required for effective downstream signaling. Third, viral infection often leads to altered expression of key micro-RNAs (miRNAs). These mechanisms prevent host cell-mediated up-regulation of defense responses, thus inhibiting the ability of mammalian cells to successfully eradicate certain viruses.

A. Strategies Based on Interference of Viral RNA Recognition

We previously stated (section IID) that one of the features that allows recognition of viral RNA by RIG-I is the presence of a 5′ppp moiety.[62,66] Viral RNAs that contain a 5′-monophosphate evade RIG-I sensing.[55] All eukaryotic cellular mRNAs are blocked at their 5′-ends with a cap structure that precludes recognition by RIG-I (section IID).[129] Many viruses use the host mRNA machinery to cap their newly synthesized mRNAs, encode their own capping enzymes, or hijack capped 5′ fragments from cellular mRNAs.[130,131] While capped viral RNAs escape RIG-I recognition, viral RNA replication leads to transient cytosolic intermediates that harbor uncapped 5′ppp, thus allowing recognition by the host.[132] Other viruses (e.g., members of the Picornaviridae and Calcioviridae families) covalently link a viral protein (VPg) to the 5′-end of viral RNAs to prevent recognition by RIG-I.[133] Finally, it is apparent that viruses such as members of the Bunyaviridae and Bornaviridae families have developed mechanisms to remove their own 5′ppp group, thus evading detection by RIG-I.[55]

A number of viruses encode cytoplasmic proteins that have the ability to shield dsRNAs to prevent sensing by RIG-I (e.g., vaccinia virus E3L, Ebola virus VP35, and influenza A virus NS1).[134-136] Interestingly, the dsRNA-binding domain of E3L inhibits 5′ppp ssRNA-, dsRNA-, and DNA-induced IFN-β synthesis.[137,138] This motif binds to dsRNA but not to ssRNA or DNA, suggesting that E3L interferes with dsRNA binding and inhibits RIG-I/MAVS signaling through dual mechanisms. Some virally encoded proteins can both sequester RNA and bind to RIG-I directly, again suggesting the use of multiple strategies to inhibit host responses.[131]

B. Strategies Based on Inhibition of RIG-I-MAVS Signaling

In addition to strategies based on structure recognition, viruses have developed equally sophisticated mechanisms to inhibit functional aspects of RIG-I/MAVS signaling. First, RIG-I cleavage is used by members of the Picornaviridae family virus to evade recognition.[128] This process involves a viral protease, 3Cpro, which has been shown to cleave RIG-I both in vitro and in vivo.[128] Second, influenza A virus NS1 binds to the RIG-I/MAVS complex and blocks downstream signaling.[136,139]

An additional mechanism of viral defense against detection involves MAVS. NS3/4A is a protease encoded by hepatitis C virus that has been known to interfere with antiviral responses before the RIG-I/MAVS signaling axis was identified.[140] It was later discovered that NS3/4A suppresses RIG-I-dependent IFN expression by cleaving MAVS from its mitochondrial tether.[31,141-144] Picornaviruses and hepatitis A virus utilize similar, protease-based defense mechanisms, suggesting that specific cleavage of MAVS may be a common strategy to dampen antiviral activity.[131,145] Other viruses (i.e., Poliovirus) trigger cleavage of MAVS through a caspase-dependent mechanism, suggesting that some viruses may activate caspases not only to facilitate shedding and replication, but also to impair antiviral responses.[146] Additional viral countermeasures in the RIG-I-MAVS pathway, especially those related to strategies involving downstream signaling molecules, are described in more detail in excellent review articles.[26,131]

C. Strategies Based on Induction of MicroRNA Expression

Novel findings indicate that viruses utilize miRNA-based strategies to evade eradication initiated by the host. miRNAs are highly conserved, non-coding RNAs that affect biological processes such as development, infection, immunity, inflammation, and tumorigenesis by suppressing gene expression through their ability to bind to the 3′-UTR of target mRNAs.[147,148] A recent interesting study by Hou et al. demonstrated that type I IFN production triggered by vesicular stomatitis virus infection is inhibited by up-regulation of miR-146a in a RIG-I-dependent manner.[149] In addition, the authors showed that pro-inflammatory cytokine and chemokine expression and NF-κB activation were also attenuated by miR-146a. These events led to the promotion of viral replication. The targets of miR-146a included TRAF6 and also IRAK (IL-1R-associated kinase)-1 and IRAK-2, two kinases shown in this work to associate with FADD.[149] Other viruses may utilize similar miRNA-based strategies to blunt type I IFN and perhaps other responses to negatively regulate host antiviral strategies.

V. Other Functions of RIG-I

RIG-I has been proposed to participate in a variety of intracellular events apparently unrelated to its role as a viral sensor. Some of these functions are summarized below.

A. Cellular Differentiation

The ability of ATRA to induce granulocytic differentiation during acute promyelocytic leukemia has been the basis for its use in the treatment of this malignancy.[150] Interestingly, ATRA-induced differentiation and normal myelopoiesis are accompanied by induction of RIG-I.[12] Conversely, disruption of the *Rig-I* gene (also known as *dxd58*) in mice leads to the development of a progressive myeloproliferative disorder.[151] These observations suggest that RIG-I may play important roles in mammalian granulocyte production and differentiation.

B. Bacterial Phagocytosis

Studies by Kong et al. revealed that, in addition to its role in antiviral responses, RIG-I participates in TLR-stimulated phagocytosis.[152] Stimulation with TLR4 ligands was shown to induce RIG-I expression in macrophages and depletion of RIG-I inhibited TLR4-induced bacterial phagocytosis. This effect seems to be mediated by activation of the small GT-Pase Cdc42/Rac1, actin polymerization, and recruitment of the actin regulator Arp2/3. Consistently, mice deficient in RIG-I expression are more susceptible to bacterial infection, suggesting that RIG-I plays an important role during antibacterial responses.[152]

C. Apoptosis

RIG-I is being actively investigated as a potential therapeutic target for cancer, particularly melanoma. Melanoma cells are known to have defects in programmed cell death in response to a wide range of external stimuli.[153] IFN-α2β, a member of the type I IFN family, is widely used as an anticancer drug against melanoma; its efficacy is significant but limited.[154] Poeck et al. designed a small RNA that silenced the anti-apoptotic gene *Bcl2* and also activated RIG-I. To accomplish this, the investigators used an elegant strategy that consisted of replacing the 5′-monophosphate ends of the synthetic siRNA with a 5′ppp. The efficacy of this approach was tested in a mouse model of metastatic melanoma to the lung.[155] Silencing *Bcl2* promoted tumor cell apoptosis, and activating RIG-I stimulated the immune system to destroy the tumor through a mechanism that involved induction of type I IFN by RIG-I.[155] These results suggest that single or combinatorial therapies that exploit the ability of RIG-I to stimulate beneficial immune responses may have potentially useful therapeutic applications for the treatment of melanoma and perhaps other types of cancer.

D. Inflammation

As previously mentioned (section IIIC), activation of the NF-κB pathway through MAVS-dependent RIG-I signaling results in the production of cytokines, including the cytokine precursor pro-IL-1β.[3] The generation of mature, biologically active IL-1β requires proteolytic processing through caspase-1, an enzyme activated by cytoplasmic multiprotein complexes known as inflammasomes.[157] Pioneering work by Rintahaka et al. demonstrated that the RIG-I signaling pathway is involved in inflammasome-associated caspase-1 activation and apoptosis of virus infected cells.[158] Poeck et al. later showed that RIG-I acts as a sensor for inflammasome activation, leading to caspase-1 activation and generation of mature IL-1β.[159] These two studies represent the first examples of MA-VS-independent RIG-I signal transduction.

VI. Regulation of RIG-I Expression

A. The *RIG-I* Promoter

In 2007, Su et al. reported the cloning and characterization of essential elements regulating expression of the human RIG-I promoter.[160] This work revealed the presence of two putative TATA boxes at positions −144 and −162 relative to the transcriptional initiation site. However, it is not clear which of these TATA boxes is functional in vivo. A canonical IRF1-binding site was identified between nucleotides −17 and −4; IRF2 was also shown to activate the RIG-I promoter.[161] As expected, binding of IRF1 to the RIG-I promoter increased following stimulation, and mutational analyses identified a specific site essential for IFN-β-mediated RIG-I induction. An uncharacterized element between nucleotides −1141 and −362 was shown to interfere with IFN-β-stimulated RIG-I promoter activity.[160] Additional experiments showed that under basal conditions, the levels of RIG-I and IRF1 are significantly higher in normal cells compared with their tumor counterparts.[160] For example, abundant expression of RIG-I was detected in normal brain, but almost no RIG-I

was present in tissues from an experimental mouse brain tumor. These results are consistent with the possibility that RIG-I may possess tumor-suppressor properties through its ability to activate IFN responses.

B. Regulation of RIG-I mRNA and Protein Expression

Under unstimulated conditions, RIG-I is expressed at relatively low levels and is thought to participate in cytoplasmic surveillance of viral nucleic acids. In addition, various agonists have been shown to upregulate RIG-I expression at the mRNA and protein level in cell culture. First, ATRA induced RIG-I expression in acute promyelocytic leukemia cells.[12] Second, using subtraction hybridization techniques, RIG-I was identified as an LPS-inducible gene expressed by human endothelial cells (section IIB1).[13] Recently, it was shown that LPS-mediated stimulation of RIG-I expression occurs through a TLR4-dependent mechanism whereby early production of IFN-β leads to the induction of RIG-I through autocrine stimulation.[162]

In addition to ATRA and LPS, type I IFNs (IFN-α and IFN-β) up-regulate RIG-I expression in a variety of cell types, including A549 human lung carcinoma cells,[61] HEK293 human kidney embryonic cells,[163] monocyte-derived dendritic cells,[164] and HeLa human cervical cancer cells.[165] IFN-γ, a member of the type II IFN family, also stimulates RIG-I expression in various cell types.[14-17,164,166-169] Interestingly, cellular exposure to poly (I:C) increases RIG-I expression in human gingival fibroblasts[170] and in human astrocytes.[171] These findings, combined with fact that poly (I:C) triggers RIG-I-mediated IFN synthesis,[70] suggest that RIG-I expression may be subject to positive feedback regulatory mechanisms. TNF-α also stimulates RIG-I expression in A549 cells,[61] HeLa cells,[165] and synoviocytes.[172] Moreover, RIG-I is synergistically induced by IFN-α and TNF-α in A549 cells.[61] Consistently, the expression of RIG-I is blocked by type I IFN receptor-specific neutralizing antibodies. In HeLa cells, RIG-I expression is mediated by IFN-ε,[165] and in synoviocytes, IFN-β seems to play a key role in RIG-I induction.[172] IL-1β up-regulates RIG-I expression in human gingival fibroblasts through an incompletely characterized mechanism.[173]

Listeria monocytogenes, an obligate intracellular parasite, infects a variety of cell types, including phagocytes.[174,175] This parasite enters host cells by interacting with eukaryotic cell-surface adhesion molecules, including E-cadherin.[174] Following internalization, parasites remain in a vacuole or escape to the cytosol, where they replicate and remain as intercellular pathogens. Imaizumi et al. found that infection with *L. monocytogenes* induces the expression of RIG-I in vitro and in vivo, and that NLRs likely mediate this effect.[176] These results suggest that RIG-I may be involved in innate immune mechanisms to protect the host against parasitic infections.[176]

VII. RIG-I in Disease

In vivo studies and work in clinical samples suggest that RIG-I may play important roles in immune responses associated with both noninfectious and infectious diseases.

A. Atherosclerosis

Atherosclerosis is best described as a chronic inflammatory condition that can be converted into an acute clinical event by plaque rupture and thrombosis.[177] The observation that IFN-γ production by proatherogenic/pro-inflammatory Th1 (T-helper 1) cells is a feature of atherosclerosis prompted Imaizumi et al. to characterize RIG-I expression in atherosclerotic plaques.[164] These studies revealed robust immunohistochemical expression of RIG-I localized to intimal macrophages recruited to human aortic lesions. Interestingly, while the monocytic cell line THP-1 displayed modest responses to IFN-γ stimulation, differentiation

into macrophage-like cells robustly increased IFN-γ-induced RIG-I expression. These studies suggest that RIG-I may play an active role in the pathogenesis of atherosclerosis through currently unknown mechanisms.

B. Psoriasis

Psoriasis vulgaris is an inflammatory disease of the skin and could arguably be considered the most prevalent cell-mediated inflammatory condition in humans.[178] Clinically, psoriatic lesions present as erythematous, sharply demarcated, raised, scaly plaques. It is generally accepted that the presence of clonal populations of T cells in these lesions represents recruitment in response to unidentified cutaneous antigens.[179,180] Interestingly, the levels of IFN-γ and RIG-I are significantly elevated in the epidermis of psoriasis patients[181] compared with normal skin cells.[182,183] It is tempting to speculate that expression of RIG-I in psoriatic lesions represents a regional immune response, and that IFN-γ may be important for this process. Future studies should characterize the functional consequences of RIG-I up-regulation in psoriatic patients. Work along these lines could generate important information to provide the basis for interventional approaches aimed at targeting this axis for the treatment of this autoimmune skin disease.

C. Rheumatoid Arthritis

Rheumatoid arthritis is a chronic autoimmune disease that affects multiple joints, and its pathogenesis is thought to involve Th1-type responses.[184] In rheumatoid arthritis, joints express modest levels of IFN-γ,[185] and mice deficient in IFN-γ receptors develop accelerated collagen-induced arthritis.[186,187] These results do not support a clear role for IFN-γ in the pathogenesis of rheumatoid arthritis, and actually suggest possible beneficial effects of this cytokine in the pathogenesis of the disease.

van Holten et al. reported high levels of IFN-β in synovial tissues from rheumatoid arthritis patients and low levels of expression in tissues from patients with osteoarthritis.[188] Interestingly, RIG-I expression mirrored that of IFN-β: that is, it was high in fibroblast-like synoviocytes from rheumatoid arthritis patients and low in tissues isolated from subjects diagnosed with osteoarthritis.[189] TNF-α, one of the main cytokines produced during the development of rheumatoid arthritis and a prime target for immunotherapy, induces RIG-I expression in rheumatoid synoviocytes in an IFN-γ-dependent fashion.[189] Further work will be required to rigorously test whether combinatorial approaches aimed at targeting IFNs or IFN-dependent signaling *and* RIG-I could be an effective therapeutic strategy for the treatment of rheumatoid arthritis.

D. Enterocolitis

The gastrointestinal mucosa is continuously being exposed to a variety of agents, including dietary antigens and microbiota. The ability of the gastrointestinal tract to distinguish pathogens from beneficial nutrients and normal microflora is a crucial homeostatic function.[190] Membrane-bound TLRs are thought to be responsible for the immunotolerance characteristic of the healthy gut. Healthy intestinal epithelial cells acquire hyporesponsiveness to the immunostimulatory activity of bacterial components by down-regulating surface TLR expression.[191,192] RIG-I is constitutively expressed on epithelial cells lining the gut mucosa,[169] and has been proposed to be important for innate antiviral responses in this organ, including inhibition of both viral replication and virus-induced apoptosis.[58] Evidence supporting a role for RIG-I in the intestine came from studies in *Rig-I*$^{-/-}$ mice. These animals are viable and fertile, but develop a colitis-like phenotype and display increased susceptibility to dextran sodium sulfate-induced colitis.[193] The phenotype is similar to that observed in G protein αi2 subunit (*Gαi2*)-deficient mice, as it is characterized by a decrease in the number and size of Peyer's patches.[193,194] In addition,

Rig-I$^{-/-}$ mice display abnormal activation of peripheral T cells and decreased expression of the G protein αi2 subunit.[193] Conversely, ATRA-induced RIG-I expression is accompanied by elevated Gαi2 levels in various tissues.[193] These results suggest possible relationships in the control of RIG-I and Gαi2 expression, and reveal a potential novel role for RIG-I in the regulation of intestinal mucosal immunity.

E. Type 1 Diabetes

Type 1 diabetes is a chronic disease resulting from the autoimmune destruction of insulin-producing β cells in the pancreas.[195] Accumulating evidence indicates that both genetic and environmental factors are important for the development of type 1 diabetes[196,197]; environmental agents involved in triggering this disease include toxins and food antigens.[197] A variety of studies indicate that viral infections also play causal roles as modulators of type 1 diabetes development through multiple mechanisms.[198] The presence of enterovirus or enteroviral RNA in the serum and in pancreatic islets of newly diagnosed type 1 diabetes in children and adults supports this possibility.[199] Studies by Dogusan et al. demonstrated that stimulation of pancreatic β cells with poly (I:C) activates IRF-3 and NF-κB, inducing production of type I IFNs that contribute to cytosolic apoptosis in a TLR3-independent process.[200] Additional work by the same group revealed that these responses occur, at least in part, through activation of RIG-I.[199] Studies aimed at identifying variants associated with altered incidence of type 1 diabetes showed that four rare variants predicted to alter the expression levels and structure of another RLR (MDA5) are independently associated with decreased incidence of type 1 diabetes.[201] These results firmly established that MDA5 is a disease-causing gene, and suggested that variants predicted to reduce MDA5 expression or activity would decrease the risk of type 1 diabetes.[201] Recent molecular and functional analyses of seven naturally occurring RIG-I variants revealed that transversion of serine 183 into isoleucine inactivates the second CARD, and results in a dominant inhibitory phenotype.[202] It remains to be determined whether this variant predisposes to type 1 diabetes, but these data, combined with the observation that attenuated function of MDA5 is associated with decreased incidence of the disease, strongly suggest that RLRs may play an important role in the pathogenesis of type 1 diabetes. Future studies should evaluate the potential utility of interventional approaches aimed at limiting expression and/or signaling through RLRs using animal models of type 1 diabetes.

VIII. Summary and Conclusions

Virologists and other scientists have dedicated much effort to the identification and characterization of mechanisms responsible for the recognition of viruses by mammalian hosts. The results of these studies led to the discovery of RIG-I and related proteins, and the elucidation of viral features required for recognition and stimulation of antiviral responses. These analyses also unveiled the existence of sophisticated downstream events necessary for signal activation and complex interactions between innate and adaptive immune responses. In addition, work from multiple laboratories provided novel information regarding the mechanisms that have evolved to optimally regulate signaling through RIG-I. Complementary analyses showed that viruses have themselves developed targeted strategies to evade detection by mammalian sensors. Continued investigations along these lines are likely to have a major impact on our ability to modulate the innate immune response so that we can effectively treat viral infections. For example, we may be able to capitalize on RIG-I- and other RLR-mediated signaling by developing specific agonists such as synthetic dsRNAs that could be used to treat viral infections and perhaps other conditions. These approaches could perhaps be complemented with inhibitors that target viral strategies known to limit host detection through RLRs. Finally, emerging functions of RIG-I that are independent of its role as a cytoplasmic viral sensor are opening new avenues for further

discovery and development of interventional approaches. For example, inhibition of RIG-I-mediated signaling has potential utility for the treatment of exacerbated immune responses, such as those typical of autoimmune disorders. Much remains to be unveiled in this exciting, relatively new field of investigation. Continued molecular studies in this area will allow us to gain deeper understanding of how the immune system orchestrates its responses, and will likely result in the identification of new strategies for the treatment of immune-related disorders and viral infections.

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Abbreviations

5′ppp	5′-triphosphate
ATRA	*all-trans* retinoic acid
CARD	caspase recruitment domain
Cardif	CARD adaptor-inducing interferon-β
DExH/D	Asp-Glu-X-His/Asp
DEAD	Asp-Glu-Ala-Asp
DDX3	DEAD box polypeptide 3
DRH	Dicer-related helicase
ds	double-stranded
DUBA	de-ubiquitinating enzyme A
FADD	Fas-associated *via* death-domain
Gαi2	G protein αi2 subunit
HSP90	heat shock protein 90
IFN	interferon
I-κB	inhibitor of NF-κB
IKK	I-κb kinase
IL	interleukin
IPS-1	IFN-β promoter stimulator-1
IRAK	IL-1R-associated kinase
IRF	interferon regulatory factor
ISG	interferon-stimulated gene
LGP	Laboratory of Genetics and Physiology
LPS	lipopolysaccharide
MAVS	mitochondrial antiviral signaling
MDA	melanoma differentiation-associated gene
miRNA	micro-RNA
NAK	NF-κB-activating kinase
NAP	NAK-associated protein

NEMO	NF-κB essential modulator
NF-κB	nuclear factor κB
NLR	NOD-like receptor
NOD	nucleotide-binding oligomerization domain
poly (I:C)	polyinosinic-polycytidylic acid
PRR	pattern-recognition receptor
RIG	retinoic acid-inducible gene
RIP	receptor-interacting protein
RISC	RNA-induced silencing complex
RLR	retinoic acid-inducible gene-like receptor
SIKE	suppressor of IKKε
SNP	single nucleotide polymorphism
ss	single-stranded
STAT	signal transducer and activator of transcription
STING	stimulator of interferon genes
TANK	TRAF family member-associated NF-κB activator
TBK	TANK-binding kinase
Th1	T-helper 1
TIM	TRAF-interacting motif
TLR	Toll-like receptor
TNF	tumor necrosis factor
TRADD	TNF receptor-associated death domain
TRAF	tumor necrosis factor receptor-associated factor
TRIM	tripartite motif
UTR	untranslated region
VISA	virus-induced signaling adaptor

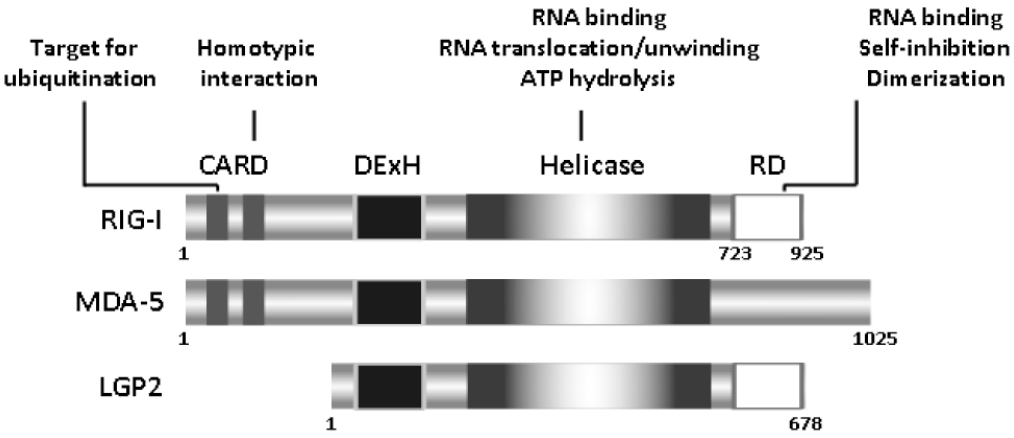

FIGURE 1.
Domain structure of RIG-I-like receptors.

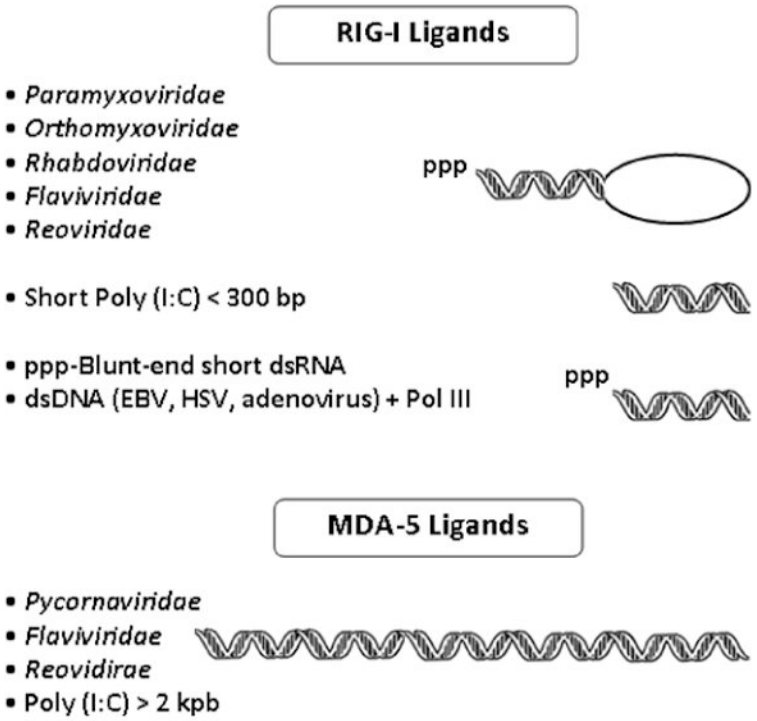

FIGURE 2.
Structural representation of ligands recognized by RIG-I and MDA5.

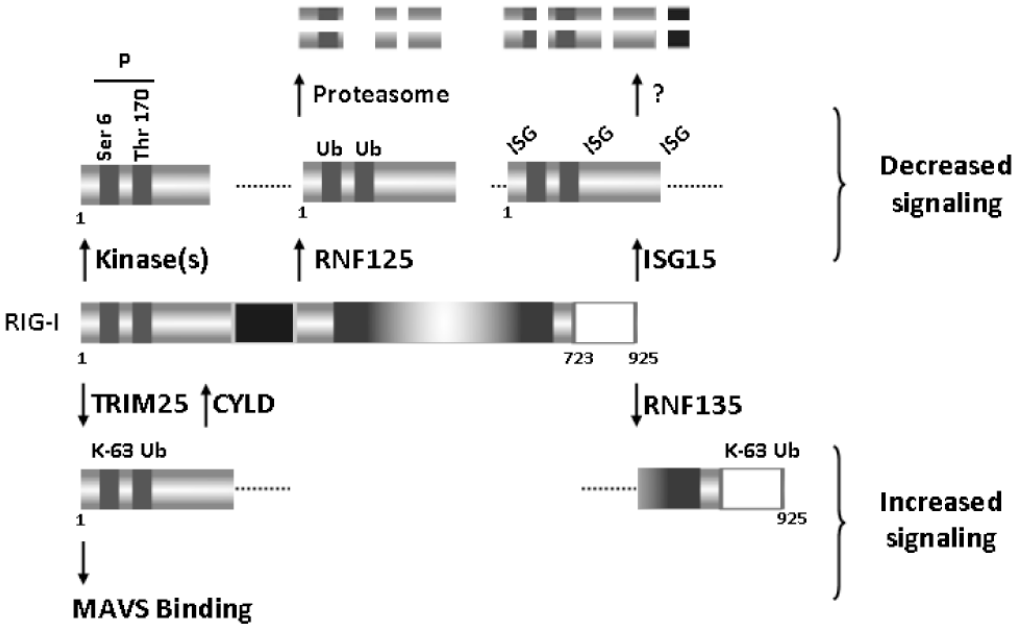

FIGURE 3.
Modulation of RIG-I activity through ubiquitin-dependent and other mechanisms.

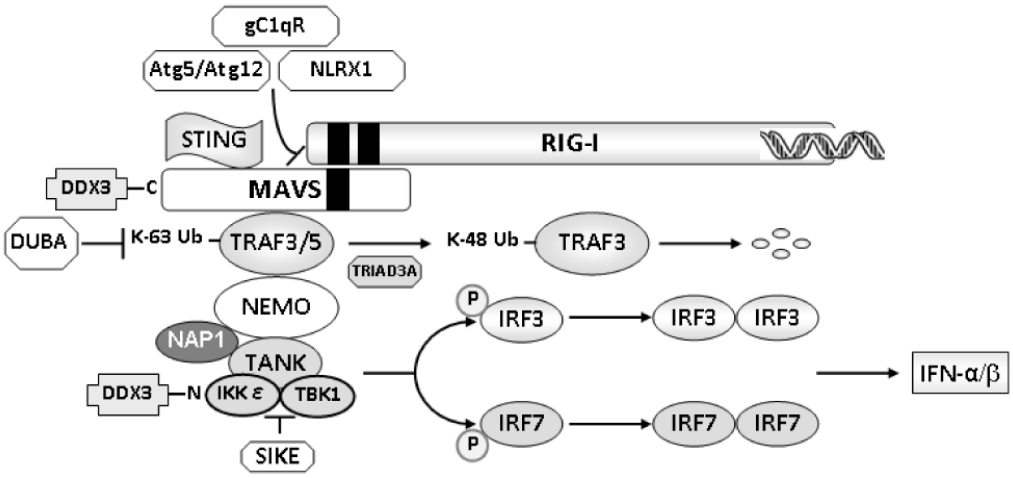

FIGURE 4.
TRAF-mediated IRF activation through noncanonical IKKs.

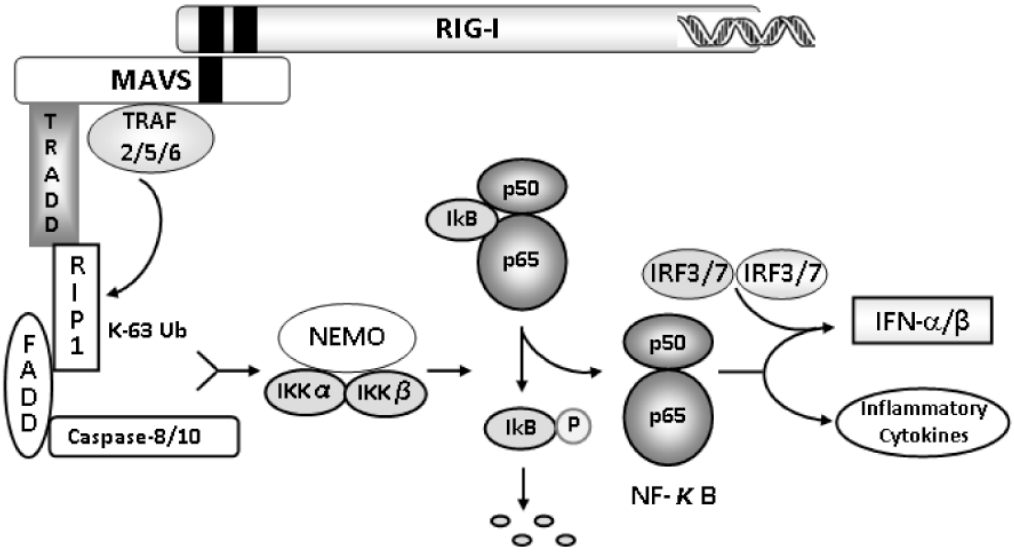

FIGURE 5.
RIG-I/MAVS signaling through the canonical IKK complex.

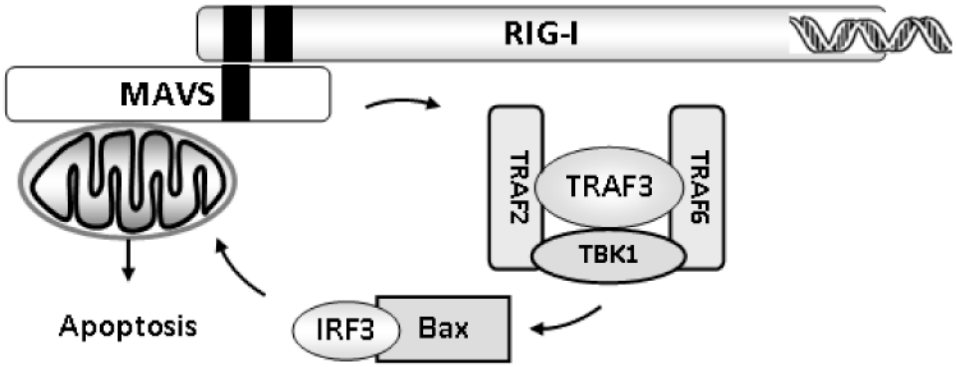

FIGURE 6.
IRF3 as a mediator of dsRNA-induced apoptosis.

TABLE 1

Viruses Sensed by RIG-I

Systematics	Virus Genome	Host	Reference
Family Herpesviridae	dsDNA		
Epstein-Barr virus		Human	60
Family Reoviridae	dsRNA		
Orthoreovirus		Human	53
Rotavirus		Zoonosis	58
Family Arenaviridae	ss(−)RNA		
Lassa virus		Rodent, human	55
Guanarito virus		Human	52,55
Sabia virus		Human	52
Machupo virus		Human	52
Junín virus		Human	52
Family Bunyaviridae	ss(−)RNA		
Rift valley fever virus		Human	
Family Filoviridae	ss(−)RNA		
Ebola virus		Human	
Family Orthomyxoviridae	ss(−)RNA		
Influenza A virus		Human	
Influenza B virus		Human	
Family Paramyxoviridae	ss(−)RNA		
Newcastle disease virus		Bird	
Sendai virus		Mouse	
Respiratory syncytial virus		Human	
Measles virus		Human	
Metapneumovirus		Human	
Nipah virus		Zoonosis	
Family Rhabdoviridae	ss(−)RNA		
Vesicular stomatitis virus		Domestic animals	
Rabies virus		Zoonosis	
Family Flaviviridae	ss(+)RNA		
Hepatitis C virus		Human	
Japanese encephalitis virus		Human	
Dengue virus		Human	
West Nile virus		Human	
GB virus/hepatitis G virus		Monkey	