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Review

Regulation of RLR-mediated innate immune signaling – It is all about keeping the balance

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ABSTRACT

The current view of cytoplasmic RNA-mediated innate immune signaling involves the differential activation of the RNA helicases retinoic acid-inducible gene 1 (RIG-I), melanoma differentiation-associated gene 5 (MDA5) and laboratory of genetics and physiology-2 (LGP2) by distinct RNA viruses. RIG-I, MDA5 and LGP2 form the RIG-I like receptor family (RLR). Since the initial characterization of the RLRs rapid progress has been made in the understanding of the molecular mechanisms that upon virus infection lead to the activation of downstream signaling cascades and the subsequent induction of type I interferon (IFN) and proinflammatory cytokines by these receptors. However, antiviral responses must be tightly regulated in order to prevent uncontrolled production of type I IFN that might have deleterious effects on the host. Exploring the structural and molecular mechanisms that underlie RLR signaling thus was accompanied by the discovery of how RLR-dependent antiviral responses are modulated. This article summarizes the current understanding of endogenous regulation in RLR signaling by various intrinsic molecules that exert their regulatory function in both the steady state or upon viral infection by targeting multiple steps of the signaling cascade.

Introduction

Vertebrates are constantly threatened by the invasion of pathogens like bacteria, fungi and parasites as well as viruses. It was Charles Janeway who in 1989 first proposed the existence of specialized pattern-recognition receptors (PRRs) that are expressed by cells of the innate immune system and that are capable of recognizing specific pathogen-associated molecular patterns (PAMPs) (Janeway, 1989). In 1996 Toll was discovered in *Drosophila* species and its mammalian homologs, the Toll-like receptors (TLRs), were found to mediate recognition of pathogens by the innate immune system (Lemaitre et al., 1996), thus fitting in Janeway's prediction of PRRs. With the discovery of the retinoic acid-inducible gene-1 (RIG-I) like receptors (RLRs) in 2005 a new pathway, which independently of the TLR system exclusively recognizes viral nucleic acids in the cytoplasm of infected cells, was identified. In total, four different classes of PRRs, including C-type lectin receptors (CLRs) and nucleotide oligomerization domain (NOD)-like receptors in addition to the TLRs and RLRs, have been found to date, the latter three being critically involved in the recognition of viral nucleic acids.

PRR-mediated antiviral signaling is rapidly induced upon viral infection, leading to the production of type I interferon (IFN) and proinflammatory cytokines. However, cytokine induction is transient and must be tightly regulated to prevent uncontrolled immune responses that might have deleterious effects to the host, promoting the development of allergy, necrosis, autoimmunity or inflammation (Theofilopoulos et al., 2005). In fact, due to the IFN-inducible nature of the RLR encoding genes, RLR-mediated production of IFN, in turn amplifies the RLR signaling pathway by upregulating RLR expression. Setting into motion the IFN-dependent amplification loop of RLR signaling, could become harmful for the host if left unchecked. Therefore, in this paper we will summarize how RLR signaling is regulated by various mechanisms and distinct proteins that are abundant in the host cell, to prevent unnecessary activation in the steady state or excessive signaling during viral infection.

Virus recognition by the RNA helicases RIG-I and MDA5

The RLRs, consisting of the three members RIG-I (Yoneyama et al., 2004), melanoma differentiation-associated gene 5 (MDA5) (Andrejeva et al., 2004) and laboratory of genetics and physiology-2 (LGP2) (Komuro and Horvath, 2006; Rothenfusser et al., 2005) are cytosolic RNA helicases, which are capable of unwinding dsRNA molecules through the hydrolysis of nucleoside triphosphates (NTPs) such as ATP (Tanner and Linder, 2001), RIG-I (DDX58) and

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MDA5 (IFIH1) belong to the family of DExD/H box containing RNA helicases, which is distinguished by the presence of several conserved motifs including the characteristic DExD/H sequence within the helicase domain, located in the central part of the molecules. In addition, both receptors possess two N-terminally located caspase recruitment and activation domains (CARDs) and a C-terminal regulatory domain (CTD) (Cui et al., 2008; Saito et al., 2007; Yoneyama et al., 2004, 2005; Zhang et al., 2000).

Although sharing a similar structure with high amino acid (aa) sequence homology, RIG-I and MDA5 were shown to play different roles in the recognition of RNA viruses (Kato et al., 2006). While RIG-I is involved in the recognition of a wide variety of RNA viruses, including paramyxoviruses, such as Newcastle disease virus (NDV) and Sendai virus (SeV), vesicular stomatitis virus (VSV), rabies virus (RV), influenza virus, ebola virus and the flaviviruses Japanese encephalitis virus (JeV) and hepatitis C virus (HCV) (Hornung et al., 2006; Kato et al., 2005, 2006; Melchjorsen et al., 2005; Spiropoulou et al., 2009; Sumpter et al., 2005), MDA5 is activated in response to infection with picornaviruses like encephalomyocarditis virus (EMCV), Mengo virus and Theiler's virus (Gitlin et al., 2006; Kato et al., 2006). In addition to the recognition of picornaviruses, MDA5 is also required for the control of murine norovirus infection, which is a surrogate model for human norovirus that causes severe viral epidemic gastroenteritis worldwide (McCartney et al., 2008).

However, recent studies revealed that some viruses, such as West Nile virus and Dengue virus as well as vaccine strains of measles virus, are redundantly sensed by both RIG-I and MDA5 (Fredericksen et al., 2008; Loo et al., 2008; Shingai et al., 2007). It seems likely that the cooperation of both RLRs has evolved as a versatile mechanism to provide maximum protection of the infected host cell to these invading viruses.

Recently, the exceptional role of RLRs in the recognition of RNA viruses was contradicted by the observation that RIG-I and MDA5 are also involved in antiviral signaling in response to viruses containing a dsDNA genome, such as Epstein–Barr virus (EBV) (Samanta et al., 2006) and vaccinia virus (Pichlmair et al., 2009), respectively (see below).

RLRs are accurate sensors of virus invasion – discrimination between self and non-self

Given that RLRs are localized in the cytoplasm, virus sensing must be highly discriminative to avoid recognition of host (self) RNAs, leading to uncontrolled autoactivation of the innate immune system in the absence of virus infection. Activation of RIG-I and MDA5 by different viruses implicated that they are specific in the detection of RNA viruses, presumably through the recognition of distinct structures of viral RNA.

In 2006, two groups independently demonstrated that RNA bearing a 5′-triphosphate structure, which is detectable in most RNA genomes as well as *in vitro* transcribed RNA, is selectively recognized by RIG-I (Hornung et al., 2006; Pichlmair et al., 2006). The current view of RIG-I activation involves that in addition to the 5′-triphosphate moiety, blunt-end base pairing at the 5′-end of the RNA is critically required (Schlee et al., 2009; Schmidt et al., 2009). Furthermore, biochemical and deep sequencing analysis revealed the exact nature of RNA molecules interacting with RIG-I during the course of SeV and influenza virus infection. Shorter RNA molecules containing 5′-triphosphorylated ends as well as some dsRNA regions preferentially associate with RIG-I. Recently, it was demonstrated that at least during the course of SeV and influenza virus infection, viral replicating RNA, not full-length genomic viral RNA, constitutes the majority of immunostimulatory RNA associated with RIG-I (Baum et al., 2010). Moreover, RIG-I is activated upon conversion of cytosolic poly(dA:dT) DNA by host RNA polymerase III into a 5′-triphosphate RNA intermediate (Ablasser et al., 2009; Chiu et al., 2009). With regard to the RIG-I-mediated recognition of the DNA virus EBV, it was shown that in latently EBV-infected cells, EBER molecules, which are small non-polyadenylated, untranslated RNA molecules of 167 nt (EBER-1) or 172 nt (EBER-2) length (Lerner et al., 1981), are transcribed in large amounts by RNA polymerase III, thus leading to the activation of RIG-I/IPS-1-mediated type I IFN induction (Ablasser et al., 2009). Activation of RIG-I by viral RNA has been linked to the concerted action of the helicase domain and the C-terminal domain (CTD) (Cui et al., 2008). The regulatory CTD (also called regulatory domain, RD) contains a conserved basic groove that specifically recognizes the RNA 5′-triphosphate structure as well as the blunt end dsRNA termini. Ligand recognition in the CTD involves multiple electrostatic interactions with the 5′-triphosphate as well as the backbone phosphodiesters of the RNA (Lu et al., 2010).

In contrast, much less is known about the nature of RNAs that are agonists for MDA5. Shortening of the synthetic dsRNA analogue poly(I:C) that mimics viral RNA converts it from a MDA5 ligand into a RIG-I ligand, suggesting that MDA5 specifically recognizes long dsRNA (Kato et al., 2008). The current view is, that activation of MDA5 requires a RNA web rather than simply long molecules of RNA (Kato et al., 2008; Pichlmair et al., 2009).

RIG-I and MDA5 share a common signaling adapter

Although RIG-I and MDA5 are involved in the recognition of a variety of different viruses and specifically discriminate their respective RNA ligands, they activate a common downstream signaling cascade that leads to the activation of interferon regulatory factor (IRF) 3 and 7 as well as nuclear factor-κB (NF-κB) and the subsequent induction of type I IFN and proinflammatory cytokines.

It was shown that mutant RIG-I lacking the caspase activation and recruitment domains (CARDs) is not capable of eliciting an antiviral response (Yoneyama et al., 2004), indicating that the CARDs act as an effector domain, which is involved in the initiation of downstream signaling. The critical role of CARD–CARD interactions for the initiation of signaling pathways was first shown for apoptosis (Vaughn et al., 1999). The CARD-containing adaptor protein that is essential for RLR-mediated antiviral signaling was then discovered by four independent groups by either BLAST search identifying CARD-containing proteins (Meylan et al., 2005; Seth et al., 2005) or cDNA library screening for activators of the IFN-β promoter (Kawai et al., 2005) and the NF-κB pathway (Xu et al., 2005). It was designated as IFN-β promoter stimulator 1 (IPS-1) (Kawai et al., 2005), mitochondrial antiviral signaling protein (MAVS) (Seth et al., 2005), virus-induced signaling adapter (VISA) (Xu et al., 2005) and CARD adapter inducing IFN-β (CARDIF) (Meylan et al., 2005). Overexpression of IPS-1 significantly enhances the RLR-triggered immune response. Conversely, in mice lacking IPS-1 the IFN response to RIG-I- and MDA5-specific virus infection is impaired (Kumar et al., 2006; Sun et al., 2006) indicating that IPS-1 functions as the essential adaptor for RLR-mediated signaling. IPS-1 contains an N-terminal single CARD as well as a central proline-rich region and a C-terminal hydrophobic transmembrane (TM) domain, which localizes IPS-1 to the outer mitochondrial membrane. Upon viral challenge, CARD–CARD-mediated receptor-adapter interaction occurs at the mitochondrium, with the second CARD of RIG-I specifically interacting with IPS-1. Mutation analyses indicated that this localization of IPS-1 is essential for its signaling function, which was abolished when IPS-1 was artificially targeted to the plasma membrane or the endoplasmic reticulum (Seth et al., 2005). Recently, it was discovered that IPS-1, in addition to the mitochondria, is localized to the peroxisomes (Dixit et al., 2010). In contrast to mitochondrial IPS-1,