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Toll-Like Receptors and RNA Helicases: Two Parallel Ways to Trigger Antiviral Responses

Review

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The early detection by the host of invading microorganisms, including viruses, depends on a limited number of specific receptors that recognize pathogen-associated molecular patterns (PAMPs). A few of these PAMPs, including ssRNA and dsRNA, are recognized by Toll-like receptors (TLR)-7/8 and TLR3, respectively. Activation of an antiviral TLR-dependent signaling cascade leads to the activation of the key transcription factors IRF and NF-κB, which promote antiviral responses through induction of specific genes. Recently, a second system has been described, which relies on the cytoplasmic recognition of dsRNA by RNA helicases such as RIG-I. In this review, we discuss the mechanistic aspects of these important arms of the host innate response to dsRNA and a few viral strategies utilized to counteract them.

Introduction

Antiviral innate immunity depends on the induction of critical proteins bearing antiviral activity. Such proteins include type-I interferons (IFN), which are represented by a single IFN-β and several IFN-α molecules. The antiviral activity of IFN was demonstrated half a century ago (Isaacs and Lindenmann, 1957) and later confirmed in IFNα/β receptor (IFNAR)-deficient mice, who show an extreme susceptibility to different viral infections (Muller et al., 1994). The induction of type-I IFN is tightly regulated and transcriptionally controlled by a limited number of transcription factors. In the case of IFN-β, an enhanceosome complex is formed by interferon regulatory factor (IRF) family members (IRF3 and IRF7 mainly), nuclear factor kappa B (NF-κB), and AP-1 (Maniatis et al., 1998). The intracellular events controlling the activity of these transcription factors have been elucidated during the recent years and constitute crucial antiviral pathways that are triggered by the detection of PAMPs derived from viruses. Viral PAMPs include double-stranded (ds) RNA and other replication intermediates. dsRNA is sensed by the host through different detection systems that trigger the antiviral immune response.

dsRNA Recognition: The Pre-TLR Era

Four decades ago, it was recognized that in addition to its presence within the genome of dsRNA viruses dsRNA could also be detected in cells infected by single-stranded (ss) RNA viruses, including the Picornaviridae family members Encephalomyocarditis virus and Poliovirus (Baltimore et al., 1964; Montagnier and Sanders, 1963). Today, dsRNA is known to represent a molecular intermediate during the viral replication of many viruses within infected cells, and therefore, it is not surprising that the host has evolved several means to detect its presence. Upon detection of dsRNA, signaling cascades promote the induction of crucial antiviral molecules, including type-I IFN. A major research focus in antiviral immunity has been, and continues to be, the elucidation of proteins involved in dsRNA-induced IFN production. Two pathways whose activities rely on dsRNA have been studied in depth. The first depends on protein kinase R (PKR). This IFN-inducible kinase becomes activated following binding to cytoplasmic dsRNA that results in the phosphorylation of eukaryotic translation initiation factor (eIF2)-α (Meurs et al., 1990). Through this mechanism, PKR is able to inhibit protein translation (and therefore virus replication) within infected cells (Williams, 2001). Further, in PKR-deficient mice, antiviral responses are diminished when coactivated by dsRNA or IFN (Durbin et al., 2002; Yang et al., 1995). However, induction of type-I IFN by dsRNA and viruses is unchanged, and neither IRF3 nor IRF7 activation is affected by the absence of PKR (Smith et al., 2001; Yang et al., 1995). These observations suggested that another molecule(s) may transmit dsRNA-dependent pathways that lead to IRF activation and subsequent type-I IFN production.

A second protein that is stimulated by dsRNA is 2′–5′ oligoadenylate synthetase, an enzyme that activates the endoribonuclease RNase L through the synthesis of short oligoadenylates. Upon activation, RNase L promotes the cleavage of both cellular and viral RNAs. Similarly to PKR, the analysis of RNase L-deficient mice revealed the requirement for this enzyme in IFN-dependent antiviral actions (Zhou et al., 1997). Thus, although both become activated by dsRNA and are implicated in antiviral immunity, PKR and RNase L are mainly IFN effectors and dispensable for IFN production. Therefore, other cellular systems explain the connection between dsRNA sensing and the upregulation of type-I IFN.

Detection of dsRNA and Signaling through TLR3

The discovery of pattern recognition receptors (PRRs) has revolutionized our understanding of innate immunity, explaining why and how multiple and diverse infectious agents are recognized by a limited number of innate immune receptors that trigger antimicrobial responses (Janeway, 1989). TLRs, numbered 1–11, are PRRs that recognize by means of their leucine-rich repeat (LRR) PAMPs derived from microorganisms. TLRs utilize their common cytoplasmic Toll-interleukin-1 receptor (TIR) domain to transmit intracellular responses through the recruitment of TIR-containing adaptors (Akira and Takeda, 2004; Janeway and Medzhitov, 2002). These adaptors include MyD88, Trif (TICAM-1), TRAM, and TIRAP (Mal), which mediate cellular events that lead to the induction of antimicrobial and inflammatory genes (O'Neill et al., 2003). Although normally present at the plasma membrane to detect extracellular PAMPs, a few TLRs, including TLR3, TLR7, TLR8, and TLR9,

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recognize their ligands in intracellular compartments such as endosomes. Interestingly, the latter TLRs share the ability of nucleic acid recognition, detecting dsRNA (TLR3), ssRNA (TLR7 in mice, TLR8 in humans), and nonmethylated CpG DNA motifs (TLR9) (Alexopoulou et al., 2001; Diebold et al., 2004; Heil et al., 2004; Hemmi et al., 2000; Lund et al., 2004). Even though these TLRs can recognize virally derived nucleic acids, they also have the propensity (at least TLR7/8 and TLR9) to interact with self nucleic acids (Barton et al., 2006; Diebold et al., 2004). Importantly, their intracellular localization may prevent them from recognizing potentially dangerous self molecules and activating signals in the absence of infection (Barton et al., 2006). These remarkable examples also suggest that recognition of pathogens and "danger" may proceed through identical receptors.

Although most TLRs recruit MyD88 to transmit antimicrobial responses, the elucidation of TLR3 signaling arose from the finding that TLR3 recruits Trif, but no other TIR adaptor, to mediate antiviral responses (Hoebe et al., 2003; Oshiumi et al., 2003a; Yamamoto et al., 2002, 2003). Trif signaling leads to IRF3 and IRF7 activation and type-I IFN production (Fitzgerald et al., 2003b; Oshiumi et al., 2003a; Yamamoto et al., 2002). To date, two kinases have been demonstrated to directly phosphorylate and activate IRF3 and IRF7: TBK1 (NAK, T2K) and IKKε (IKKi) (Bonnard et al., 2000; Fitzgerald et al., 2003a; Peters et al., 2000; Pomerantz and Baltimore, 1999; Sharma et al., 2003; Shimada et al., 1999; Tojima et al., 2000). Although the contribution of IKKε in TLR3 signaling is of debate, TBK1 can be recruited to the N-terminal region of Trif to activate IRF (Figure 1). Trif-induced NF-κB activation may proceed through two ways. First, Trif recruits tumor necrosis factor receptor (TNF-R) associated factor (TRAF)-6 by means of its N-terminal region (Jiang et al., 2003; Sato et al., 2003). However, the significance of TRAF6 in this context is still unclear due to the potential redundancy provided by other TRAF molecules (Gohda et al., 2004; Hacker et al., 2006). Second, Trif contains a C-terminal RIP homotypic interaction motif (RHIM; [Sun et al., 2002]) that interacts with the RHIM of both receptor interacting protein (RIP)-1 and RIP3 kinases (Meylan et al., 2004). Whereas RIP1 transmits Trif-induced NF-κB activation, RIP3 appears to bear regulatory functions (Figure 1) (Cusson-Hermance et al., 2005; Meylan et al., 2004). However, the role of RIP3 in this context requires examination in RIP3-deficient animals, which have no apparent phenotypic alteration (Newton et al., 2004). In addition to these findings, other molecules were proposed to interact with the adaptor Trif and to modulate signaling (Table 1). Interestingly, two recent studies have used TRAF3-deficient cells to reveal this molecule as an essential intermediate transmitting antiviral responses, partly through binding to Trif and the kinases TBK1 and IKKε (Hacker et al., 2006; Oganesyan et al., 2005). These exciting studies lay the groundwork to provide a clear function for this TRAF member and raise many questions. For example, whether Trif recruits both IRF3 kinases through a TRAF3-dependent manner, or whether TRAF3 forms a secondary complex containing Trif, TBK1, IKKε, and possibly other proteins, remains to be investigated. Further, that TRAF3 bears ubiquitin ligase activity to trigger ubiquitination of signaling molecules, similar to TRAF6 (Sun et al., 2004), is an intriguing proposition. Hence, TLR3 fulfills antiviral innate immune functions by connecting dsRNA recognition to the activation of crucial transcription factors.

Interestingly, TLR3 can also promote crosspriming of cytotoxic T lymphocytes (CTLs). In dendritic cells that are not infected by viruses per se (and therefore that do not process viral antigens to directly present them to CTL), TLR3 can recognize dsRNA from phagocytosed apoptotic cells that had been previously infected (Schulz et al., 2005). This recognition promotes CTL responses, most probably through the induction of costimulatory signals by noninfected dendritic cells.

Of note, the importance of TLR3 as a truly antiviral receptor was recently challenged in vivo because wild-type and TLR3-deficient mice exhibit a similar susceptibility to several viruses, including vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), reovirus, and murine cytomegalovirus (MCMV) (Edelmann et al., 2004; Schroder and Bowie, 2005). These results have raised the possibility that other, TLR3-independent, mechanisms may trigger antiviral responses. In-depth analysis of TLR7-deficient mice has revealed increased sensitivity to different viruses such as VSV and has permitted to define this TLR as a prominent sensor of viral ssRNA located within endosomes of plasmacytoid dendritic cells (pDCs) (Diebold et al., 2004; Heil et al., 2004; Lund et al., 2004). To promote type-I IFN production in pDCs, TLR7 most probably utilizes MyD88, IL-1R associated kinase (IRAK)-1, TRAF3 and TRAF6 (Figure 1) (Hacker et al., 2006; Kawai et al., 2004; Oganesyan et al., 2005; Uematsu et al., 2005). In addition, the recent characterization of RNA helicases that act as cytoplasmic receptors has elucidated how intracellular dsRNA triggers antiviral responses.

Detection of Cytoplasmic dsRNA by the RNA Helicases RIG-I and MDA5/Helicard

The recent characterization of retinoic acid inducible gene-I (RIG-I) as a cytoplasmic dsRNA receptor has defined a novel antiviral pathway and has also permitted a clearer comprehension of signals emanating from TLR-dependent and -independent antiviral mechanisms (Yoneyama et al., 2004). RIG-I, whose expression is inducible by retinoic acid, IFN, and viral infection, is a member of the DExD/H box RNA helicases (Zhang et al., 2000). Human RIG-I encodes for a protein of 925 amino acids that contains an N-terminal region characterized by the presence of two caspase recruitment domains (CARD) and a C-terminal region harboring potential ATP-dependent RNA helicase activity (Figure 1). CARDs are members of the death domain fold family that also includes death domains (DDs), death effector domains (DEDs), and pyrin domains (PYDs). These modules are composed of six antiparallel α helices and are implicated in homotypic interactions (a CARD always interacts with another[s] CARD[s] for example) related to inflammatory or cell death pathways (Hofmann, 1999; Martinon et al., 2001).

RIG-I activates NF-κB and IRF3 through its CARD-containing N-terminal region and inhibits VSV- and EMCV-induced infectivity when stably expressed in cells (Yoneyama et al., 2004). In contrast, mutant forms of RIG-I, either deleted of the N-terminal region or rendered catalytically inactive, both act as dominant negatives by diminishing dsRNA- and Newcastle disease virus