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REVIEW

Innate immune recognition of viral infection

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Induction of the antiviral innate immune response depends on recognition of viral components by host pattern-recognition receptors. Members of the Toll-like receptor family have emerged as key sensors that recognize viral components such as nucleic acids. Toll-like receptor signaling results in the production of type I interferon and inflammatory cytokines and leads to dendritic cell maturation and establishment of antiviral immunity. Cells also express cytoplasmic RNA helicases that function as alternative pattern-recognition receptors through recognition of double-stranded RNA produced during virus replication. These two classes of pattern-recognition receptor molecules are expressed in different intracellular compartments and induce type I interferon responses via distinct signaling pathways.

Viral infection remains a considerable health threat. This was not always considered to be the case. The development of antibiotics and vaccinations brought optimism to many people that infectious diseases might be easily eradicated. The emergence of new diseases such as acquired immune deficiency syndrome, severe acute respiratory syndrome and avian influenza, along with the resurgence and expansion of existing diseases, including West Nile and Ebola hemorrhagic fevers, effectively destroyed such optimism. Moreover, although antiviral drugs that inhibit replication of viruses are now available, many viruses, including human immunodeficiency virus (HIV), mutate readily and produce resistant strains that are no longer controlled by drugs that were once effective. Furthermore, with increasing global transportation of goods and people, viral infections that would have been otherwise confined to a limited geographic region can now easily spread worldwide. The prospect of biological warfare by terrorists is another serious new threat. All of these circumstances present challenges requiring the development of new strategies aimed against life-threatening viruses. To achieve this, more must be learned about the mechanisms of host-virus interactions, including antiviral responses exerted by host immune systems, as well as defense mechanisms of viruses that permit them to evade host immune responses. In this review we discuss recent progress in understanding antiviral host responses of the innate immune system.

Antiviral immune response

The innate immune recognition of virus infection triggers antiviral immune responses[1,2]. Virus-associated molecules such as genomic DNA and RNA or double-stranded RNA (dsRNA) produced in virally infected cells can be recognized by host pattern-recognition receptors (PRRs) expressed in innate immune cells such as dendritic cells (DCs)[1,2]. After recognition of viral components, PRRs initiate effective and appropriate antiviral responses, including production of a variety of cytokines and induction of inflammatory and adaptive immune responses[1–7]. In particular, type I interferons (both IFN-β and the multiple IFN-α molecules) are the key cytokines produced after viral infection that mediate induction of both the innate immune response and the subsequent development of adaptive immunity to viruses[1,2,8]. Type I interferons induce maturation of DCs by increasing expression of costimulatory molecules such as CD80, CD86 and CD40, as well as antigen presentation via major histocompatibility complex class I, which, in addition to classical endogenous antigen presentation, facilitates cross-presentation of viral antigens[2]. Type I interferons also mediate induction of antigen-specific $CD8^+$ T cell responses[2] and chemokines, which cause stimulation and recruitment of lymphocytes and monocytes to inflamed sites. In addition to the modification of innate and adaptive immunity, type I interferons also upregulate hundreds of effector molecules that directly influence protein synthesis, cell growth and survival in the process of establishing an 'antiviral state'[2].

Expression of type I interferons is transcriptionally regulated through coordinated activation of latent transcription factors including NF-κB, ATF2–c-Jun, interferon-regulatory factor 3 (IRF3) and IRF7 (ref. 9). Of these factors, IRF3 and IRF7 in particular are activated in response to viral infection and are mainly involved in type I interferon induction. In contrast, NF-κB and ATF2–c-Jun are activated in response to various stimuli to regulate the expression of genes relevant to inflammation[10], though they also participate in enhancing type I interferon induction through cooperative interactions with IRF3 and IRF7 (ref. 9).

Toll-like receptors (TLRs) function as PRRs that principally sense conserved molecular motifs called 'pathogen-associated molecular patterns' (PAMPs) found in a wide variety of pathogens, including bacteria, fungi, protozoa and viruses[2,3,6,7]. Several TLRs expressed on plasmacytoid DCs (pDCs) participate in the recognition of viral components, such as genomic DNA and RNA, in a replication-independent way[1,11–16]. Additionally, cells express intracellular RNA helicases that function as PRRs of actively replicating viruses[17,18]. These PRRs are also essential in establishing antiviral immunity by triggering type I interferon responses.

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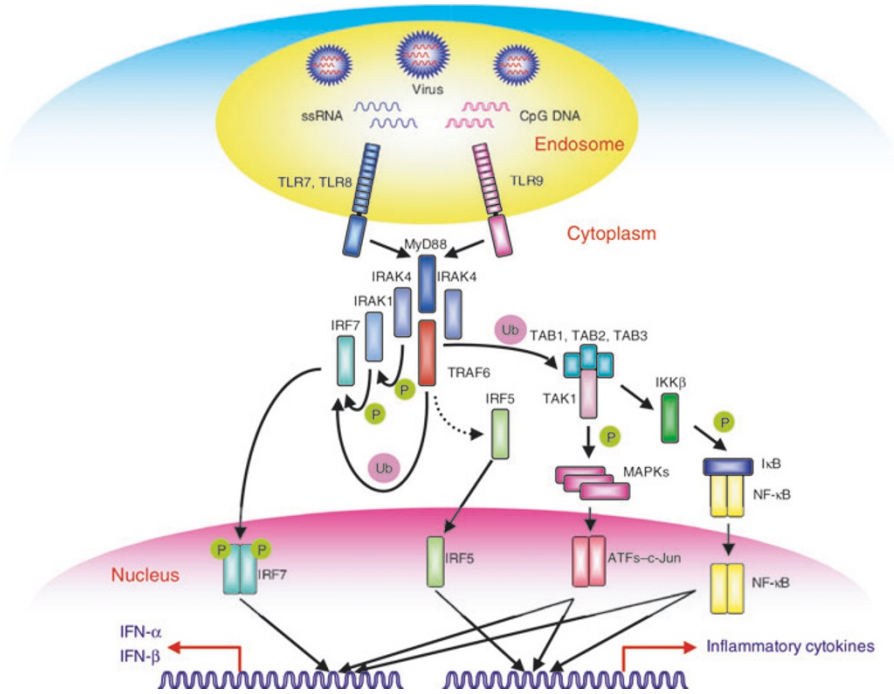

Figure 1 Recognition of viral nucleic acids by TLR7, TLR8 and TLR9 in pDCs. Recognition of viral nucleic acids by endosomal TLR7, TLR8 and TLR9 on pDCs leads to recruitment of the adapter MyD88, which in turn interacts with IRAK4 and TRAF6. TRAF6 activates TAK1 in a ubiquitination (Ub)–dependent mechanism. TAK1, together with TAB1, TAB2 and TAB3, activates NF-κB and ATF2–c-Jun via canonical IKK-mediated IκB degradation and MAP kinase (MAPK) activation, respectively. This pathway mainly controls the expression of inflammatory cytokines. MyD88 also associates with IRAK1 and IRF7. IRF7 is phosphorylated (P) by IRAK1 and translocates to the nucleus. TRAF6-mediated ubiquitination (Ub) is also required for IRF7 activation. This pathway regulates expression of type I interferon.

TLR7, TLR8 and TLR9 in pDCs

pDCs, also known as interferon-producing cells, are a restricted subset of DCs with a plasmacytoid morphology that are specialized in secreting copious type I interferon, particularly IFN-α, after stimulation with viral nucleic acids[19,20]. Both human and mouse pDCs express TLR7 and TLR9 but not TLR2, TLR3, TLR4 or TLR5 (refs. 1,19,20). Consistent with this restricted expression pattern, pDCs do not respond to bacterial components such as lipopolysaccharide and peptidoglycan, which are the ligands for TLR4 and TLR2, respectively[1,19,20]. TLR7 and TLR9 represent a structurally related subfamily that responds to nucleic acids by eliciting type I interferon production. TLR7 recognizes ribonucleic acid homologs such as imiquimod, resiquimod (R-848) and loxoribine, and synthetic single-stranded RNA (ssRNA) oligonucleotides rich in guanosine or uridine derived from ssRNA viruses such as HIV and influenza virus[11–14]. Accordingly, IFN-α production by pDCs from TLR7-deficient mice is impaired after infection with influenza virus or vesicular stomatitis virus (VSV)[12,14]. In addition, nonviral synthetic poly-uridine RNA and some silencing RNAs also activate pDCs via TLR7 (refs. 12,21). TLR8 is phylogenetically close to TLR7. Reconstitution experiments have demonstrated that human TLR8, like TLR7, can mediate recognition of HIV-derived ssRNA and R-848 (refs. 12,22). However, TLR8-deficient mice respond normally to these molecules, suggesting species-specific functions for TLR8 (ref. 13).

TLR9 recognizes unmethylated 2′-deoxyribo (cytidine-phosphate-guanosine) (CpG) DNA motifs that are present in bacterial and viral DNA[23]. Synthetic CpG oligonucleotides have a strong ability to promote T helper type 1 immune responses and are used as a vaccine adjuvant or an anti-allergy agent. Notably, synthetic CpG oligonucleotides are also capable of triggering IFN-α production by pDCs in a TLR9-dependent way[15,16,24,25]. Analyses of pDCs derived from TLR9-deficient mice have shown that TLR9 is required for IFN-α production in response to DNA viruses such as mouse cytomegalovirus (MCMV), herpes simplex virus 1 (HSV-1) and HSV-2 (refs. 15,16,25). Inactivated HSV-2 or purified HSV-2 genomic DNA can also trigger IFN-α production by pDCs in a TLR9-dependent way[16]. Unlike the other TLRs, which are expressed mainly on cell surfaces and recognize bacterial components, TLR7, TLR8 and TLR9 are located exclusively in endosomal compartments[1,26]. Treatment of pDCs with lysosomal inhibitors such as chloroquine efficiently abrogates TLR7, TLR8 and TLR9 responses[12,14,16]; thus, viral nucleic acids are likely to be delivered to the endosomal compartment after endocytosis, where these TLRs recognize the released nucleic acids to trigger intracellular signal transduction.

Signaling via TLR7, TLR8 and TLR9

TLRs contain N-terminal extracellular leucine-rich repeats that mediate recognition of PAMPs, followed by a transmembrane domain and a cytosolic Toll–interleukin-1 (IL-1) receptor (TIR) homology domain that is required for intracellular signaling[3,7,27]. After recognition of PAMPs, TLRs recruit a set of TIR-containing adaptors including MyD88, TIRAP (also called Mal), TRIF (also called TICAM1) and TRAM[3,27]. MyD88, used by most TLRs except for TLR3 (refs. 3,27), forms a complex with members of the IL-1 receptor–associated kinase (IRAK) family (IRAK1 and IRAK4) and TRAF6 (refs. 3,27). TRAF6 functions as the K63-linked ubiquitin E3 ligase to activate the protein kinase TAK1 (refs. 28,29). Together with TAB1, TAB2 and TAB3, TAK1 activates the canonical IκB kinases (IKKs) IKKα and IKKβ, which leads to phosphorylation and subsequent degradation of IκB, allowing nuclear factor NF-κB to translocate into the nucleus[27,30,31]. TAK1 simultaneously phosphorylates two members of the mitogen-activated protein (MAP) kinase kinase family, MKK3 and MKK6, which subsequently phosphorylate c-Jun N-terminal kinases and p38 to activate ATF2–c-Jun[27]. Consistent with that, TAK1-deficient B cells show reduced activation of NF-κB and MAP kinases in response to CpG DNA[32]. The activation of this pathway, referred to as the 'MyD88-dependent pathway,' controls the expression of the genes required for inflammatory and adaptive immune responses, such as IL-6, IL-1β, tumor necrosis factor (TNF) and IL-12p40 (refs. 27,33–35; **Fig. 1**).

TLR7, TLR8 and TLR9 also elicit type I interferon induction, especially that of IFN-α, through MyD88 (refs. 24,36). In pDCs, MyD88 forms a molecular complex with IRF7 (refs. 37,38). Although IRF7-deficient pDCs are incapable of producing IFN-α in response to CpG DNA, HSV-1 or VSV, IRF3 is dispensable for IFNα production by these three TLR ligands[39]. Notably, unlike other types of DCs, pDCs have high expression of IRF7, which may explain the ability of pDCs to rapidly produce type I IFN[40,41]. IRF7 is phosphorylated by IRAK1 in response

to CpG DNA and translocates into the nucleus[42]. IRAK1-deficient mice are thus unable to produce IFN-α and activate IRF7 in response to TLR7, TLR8 and TLR9 ligands[42]. Furthermore, although activation of NF-κB and IRF7 is impaired in MyD88-deficient and IRAK4-deficient mice, NF-κB activation is relatively normal in IRAK1-deficient mice[38,42]. Thus, IRAK1 specifically mediates IRF7 activation 'downstream' of MyD88 and IRAK4. Finally, IRF7 activation requires TRAF6 E3 ubiquitin ligase activity[37], although the function of TRAF6-mediated ubiquitination in this signaling pathway remains unclear (**Fig. 1**).

Studies have produced new insights into the mechanisms of how pDCs, but not other types of conventional DCs (cDCs), produce IFN-α production after CpG DNA stimulation[43]. For example, A/D-type CpG DNA, which causes IFN-α production by pDCs, localizes together with TLR9, MyD88 and IRF7 in the endosomal compartment in pDCs, whereas it is not retained in endosomes but exclusively localizes in lysosomes in cDCs. However, when A/D-type CpG DNA is manipulated into endosomes of cDCs with the use of a cationic lipid such as DOTAP, these cells produce large amounts of IFN-α through activation of the MyD88-IRF7 pathway. B/K-type CpG DNA, which does not induce IFN-α production in pDCs, also produces similar effects if it is manipulated into endosomes[43]. These observations suggest that pDCs have a unique mechanism that can retain the CpG DNA-TLR9 complex in endosomes, which are required for robust IFN-α production.

In contrast to the critical function of IRF7 in type I interferon induction, IRF5 is required for proinflammatory cytokine induction rather than type I interferon induction by TLR3, TLR4, TLR7 and TLR9 (ref. 44). IRF5 associates with MyD88 and translocates to the nucleus in response to ligand stimulation of TLRs. In the nucleus, IRF5 binds typical interferon-stimulated response element motifs in promoter regions of cytokine genes, causing them to be expressed. The finding that IRF5 is required for TLR3 signaling, whereas MyD88 is dispensable, suggests a previously unknown mechanism of MyD88-independent IRF5 activation[44]. Conversely, human IRF5 is suggested to selectively participate in TLR7-mediated type I interferon induction rather than proinflammatory cytokine induction[45]. The mechanisms of the functional differences between human and mouse IRF5 remain unclear.

Recognition of dsRNA by TLR3

dsRNA, which is produced by many viruses during replication, is a common viral PAMP, and a synthetic analog of viral dsRNA, polyinosinic acid–cytidylic acid (poly(I)•poly(C)), has been extensively used to mimic viral infection. Induction of type I interferon, interferon-inducible genes and proinflammatory cytokines by poly(I)•poly(C), or genomic RNA purified from reovirus, are reduced in cells from TLR3-deficient mice[46], indicating that TLR3 responds to poly(I)•poly(C) and, more generally, to dsRNA. Accordingly, TLR3-deficient mice are susceptible to MCMV infection because of reduced interferon production[47]. However, TLR3-dependent recognition of virus is like a 'double-edged sword' in the case of West Nile virus infection[48], because this ssRNA flavivirus induces inflammatory responses in a TLR3-dependent way that trigger a breakdown of the blood-brain barrier, which itself results in enhanced brain infection[48]. In agreement with those results, TLR3-deficient mice survive otherwise lethal West Nile virus infection because of reduced virus entry into the brain, supporting the conclusion that TLR3-induced inflammatory responses to West Nile virus contribute to pathogenesis rather than to protection.

Signaling through TLR3 induces DC activation and promotes cross-priming of T cells, which are necessary for the induction of virus-specific T cell responses. The process by which this occurs is phagocytosis of apoptotic bodies of virus-infected or dsRNA-loaded cells by $CD8\alpha^+$ DCs that have high expression of TLR3. The dsRNA in the apoptotic bodies is recognized by TLR3, triggering the maturation of immature $CD8\alpha^+$ DCs that are required for subsequent induction of antigen-specific $CD4^+$ and $CD8^+$ T cell responses[49]. In addition, type I interferons released from virus-infected cells facilitate cross-priming[49]. Thus, a TLR3-dependent pathway mediates both innate and adaptive immune responses.

Cellular localization of TLR3 differs depending on cell type. TLR3 localizes to intracellular vesicles in cDCs, whereas it is expressed on the cell surface of fibroblasts[50]. The TLR3-containing vesicle in cDCs is thought to consist of endosomes, because inhibition of endosomal acidification abrogates TLR3-dependent poly(I)•poly(C) responses. However, localization of TLR3 and endosomes together has not been reported[50].

TLR3-mediated signaling pathway

Signaling through TLR3 activates IRF3 and NF-κB via the adaptor molecule TRIF[51–54] (**Fig. 2**). In response to the stimulation, IRF3 is

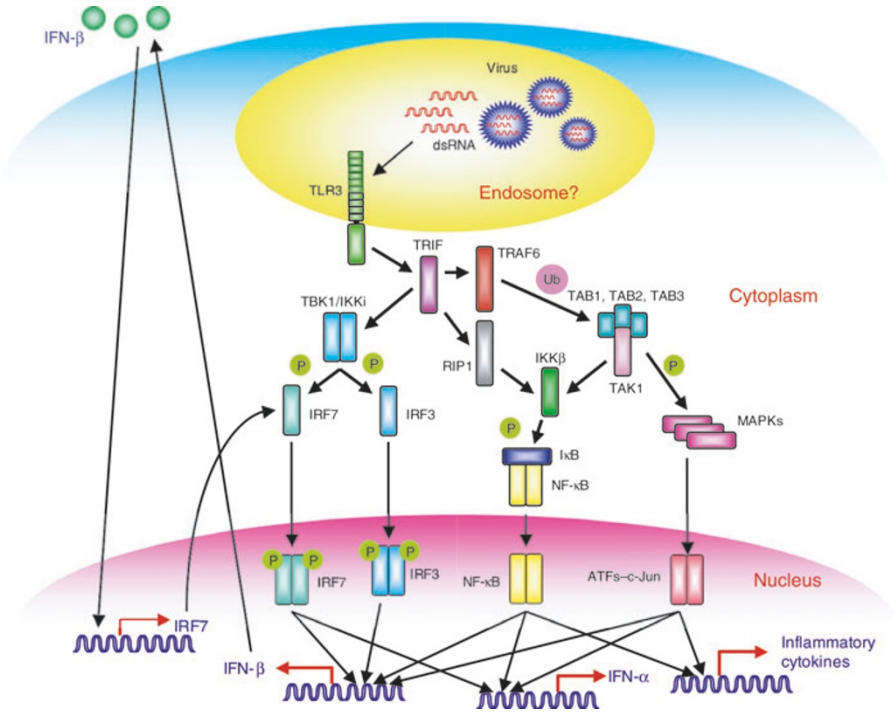

Figure 2 TLR3-dependent signaling pathway. After recognizing extracellular dsRNA, TLR3 transmits signals through TRIF, which interacts with TBK1, RIP1 and TRAF6. TBK1, together with IKKi, phosphorylates (P) IRF3, allowing IRF3 to translocate into the nucleus and activate type I interferon promoters, particularly the IFN-β promoter. Secreted IFN-β stimulates expression of IRF7, which induces IFN-α probably through a TBK1–IKKi–dependent mechanism. Both TRAF6 and RIP1 are involved in the activation of NF-κB. TAK1 is activated by TRAF6-dependent ubiquitination and mediates NF-κB and MAP kinase activation.

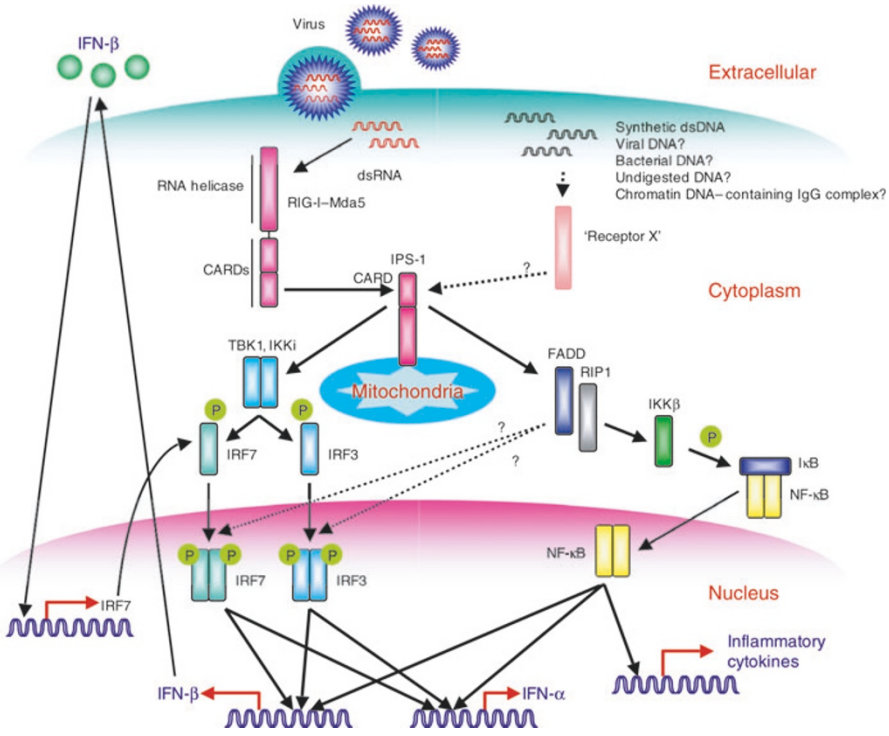

Figure 3 Signaling pathway triggered by RIG-I, Mda5 and DNA. Viruses entering the cytoplasm produce dsRNA during replication. RIG-I and Mda5 recognize dsRNA to initiate antiviral signaling. IPS-1 interacts with RIG-I and Mda5 via the CARD-like domain, followed by the activation of IRF3 and IRF7 via TBK1- and IKKi-dependent phosphorylation (P). IPS-1 also activates NF-κB via FADD- and RIP1-dependent pathways. These pathways coordinately activate type I interferon promoter. Synthetic dsDNA also activates an IPS-1-dependent pathway, although a receptor responsible for DNA recognition has not been identified. It is possible that such a putative sensor would recognize bacterial DNA, viral DNA, undigested DNA and chromatin DNA–containing immunoglobulin G (IgG) complexes.

phosphorylated by noncanonical IKKs, TBK1 (also called NAK or T2K) and IKKi (also called IKKε) and then translocates into the nucleus[55,56]. Whereas fibroblasts prepared from TBK1-deficient mice show decreased IRF3 activation and IFN-β induction by poly(I)•poly(C), IKKi-deficient cells show normal IRF3 activation[57–59]. However, residual activation of IRF3 found in TBK1-deficient cells is completely abolished in cells doubly deficient in TBK1 and IKKi[58], indicating that the functions of TBK1 and IKKi are redundant. TRIF also interacts with TBK1, indicating that TRIF links TLR3 to TBK1 (ref. 60) (**Fig. 2**). TBK1 and IKKi can phosphorylate IRF7 in addition to IRF3 (ref. 55), and although IRF3 is ubiquitously expressed, IRF7 is expressed weakly in most cell types but is transcriptionally upregulated after either virus infection or treatment with type I interferon. Thus, IRF3 is involved in a primary IFN-β and IFN-α4 induction. These subtypes of interferon subsequently activate a transcriptional complex known as ISGF3 (which consists of transcriptional activators STAT1 and STAT2, and IRF9) through the type I interferon receptor, stimulating the expression of the interferon-inducible genes, including IRF7, required for antiviral responses (ref. 9). TBK1 and IKKi phosphorylate IRF7 in a way similar to IRF3, inducing type I interferon. Thus it is also possible that IRF3 in concert with IRF7 participates in the robust induction of type I interferon through a positive feedback mechanism.

TLR3-mediated NF-κB activation is also triggered by a TRIF-dependent mechanism. The C-terminal, non-TIR region of TRIF associates with RIP1 through the RIP homotypic interaction motif[61]. RIP1-deficient cells fail to activate NF-κB in response to poly(I)•poly(C), whereas IRF3 activation is intact[61]. Through N-terminal TRAF6-binding motifs, TRIF also interacts directly with TRAF6; consistent with that, overexpression of dominant negative TRAF6 blocks TRIF-induced NF-κB activation[60]. However, NF-κB activation by poly(I)•poly(C) is abrogated in TRAF6-deficient fibroblasts, whereas NF-κB activation occurs normally in TRAF6-deficient macrophages[62], suggesting a cell type–specific function of TRAF6 in NF-κB activation. TAK1 is also involved in TLR3-mediated NF-κB and MAP kinase activation[32]. Moreover, although TAK1 is activated by TRAF6 in a ubiquitin-dependent way, it is unclear whether TAK1 is also involved in RIP1-dependent NF-κB activation (**Fig. 2**).

Recognition of virus by RNA helicases

It is becoming increasingly apparent that there are TLR-independent mechanisms of virus sensing. TLR3 is reportedly dispensable for pathogenesis and adaptive immune responses in MCMV, VSV, lymphocytic choriomeningitis virus and reovirus infections[63]. Fibroblasts or cDCs derived from TLR3-deficient mice are capable of producing IFN-β after intracellular administration of poly(I)•poly(C) and infection with Sendai virus, Newcastle disease virus and VSV[17,58,64]. Furthermore, TLR3-independent DC maturation and induction of cross-priming by dsRNA has also been suggested[49]. In pDCs, respiratory syncytial virus infection triggers type I interferon induction independent of MyD88 but entirely dependent on virus entry into cytosol and replication[65]. Also, no notable increase in HSV-1 replication is found in TLR9-deficient mice after local infection[25]. Those observations collectively suggest that host cells express additional receptors that detect actively replicating viruses in cytoplasm.

The cytoplasmic protein retinoic acid–inducible gene I (RIG-I) was identified as participating in the recognition of virus nucleic acid inside the cell, thus providing initial understanding of the alternative pathway of virus detection[17]. RIG-I is a DExD/H box RNA helicase that has two caspase-recruiting domain (CARD)–like domains, and although the helicase domain is required for interaction with dsRNA, it is the CARD-like domains that are responsible for activating 'downstream' signaling leading to IRF3 and NF-κB[17]. The data for functional importance of RIG-I include overexpression of RIG-I, which blocks replication of VSV and encephalomyocarditis virus, and 'knockdown' of RIG-I with small interfering RNA, which reduces type I interferon production after infection with Newcastle disease virus, Sendai virus and encephalomyocarditis virus[17]. In addition, cDCs and fibroblasts derived from RIG-I-deficient mice are almost completely unable to produce type I interferon and inflammatory cytokines after infection with Newcastle disease virus, Sendai virus or VSV[18]. Melanoma differentiation-associated gene 5 (Mda5; also called Helicard) is structurally related to RIG-I, as it contains two CARD-like domains and a single helicase domain[66–69]. Moreover, as with RIG-I, overexpression of Mda5 enhances Newcastle disease virus–induced type I interferon production as well as antiviral responses to infection with VSV or encephalomyo-

carditis virus[69], whereas 'knockdown' of the gene encoding Mda5 blocks Newcastle disease virus–induced activation of type I interferon promoters[69]. An RNA helicase LGP2 is also related to RIG-I and Mda5, but it lacks the CARD-like domains and is therefore suggested to be a negative regulator of RIG-I of Mda5 (refs. 69,70).

IPS-1, an adaptor triggering antiviral response

Embryonic fibroblasts deficient for both TBK1 and IKKi are unable to produce IFN-β in response to intracellular administration of dsRNA and viral infection, suggesting that both TLR3- and RIG-I–Mda5–dependent pathways converge at TBK1 and IKKi[58]. The death domain–containing proteins FADD and RIP1, which were originally identified to associate with the TNF receptor family of death receptors, are required for type I interferon induction by intracellular dsRNA stimulation[71]. In drosophila, FADD and RIP1 are essential components of the Imd pathway, which mediates antibacterial innate immune responses[72]. Drosophila FADD and RIP1 activate Relish (a homolog of NF-κB) through activation of DREDD (a homolog of caspase 8)[72]. Together, FADD and RIP1 probably form a molecular complex that mediates antiviral as well as TNF-mediated responses in mammals. However, direct interaction between RIG-I and TBK1, IKKi, FADD or RIP1 has not been reported. Given that RIG-I and Mda5 contain CARD-like structures, it is possible that adaptors containing a similar CARD-like structure participate in this pathway. IPS-1 has been identified as being involved in this pathway[73]. IPS-1 contains a CARD-like domain that mediates interaction with RIG-I and Mda5 (ref. 73). Overexpression of IPS-1 activates IFN-β, IFN-α4, IFN-α6 and NF-κB promoters and inhibits VSV replication, and 'knockdown' of the gene encoding IPS-1 blocks interferon production in response to intracellular administration of dsRNA or infection with VSV or Newcastle disease virus[73]. Furthermore, that same report shows that IPS-1-dependent type I interferon induction requires TBK1 and IKKi, although it does not directly bind to these protein kinases. IPS-1 interacts with FADD and RIP1 via its non-CARD region to facilitate NF-κB activation[73]. The evidence suggests that IPS-1 is an adaptor linking RIG-I and Mda5 to 'downstream' signaling mediators including FADD, RIP-1, TBK1 and IKKi (**Fig. 3**).

Mitochondrial antiviral signaling protein (MAVS), virus-induced signaling adaptor (VISA) and CARD adaptor inducing IFN-β (Cardif) have been identified through a database search on the basis of the similarity to the CARD-like domain of Mda5 and RIG-I. It was soon realized that MAVS, VISA, Cardif and IPS-1 represent the same protein (refs. 74–77). One additional intriguing fact reported in those findings is that MAVS localizes to mitochondria via its C-terminal hydrophobic region. Functional studies have indicated that the mitochondrial retention of MAVS is required for signaling, suggesting that mitochondria may somehow be important in antiviral immune responses (such as through induction of apoptosis).

Viral evasion of TLR and RIG-I/Mda5 signaling

Many viruses seem to encode specific proteins that inhibit the function of type I interferon. The vaccinia virus–encoded A46R protein contains a TIR domain. A46R interacts with multiple TIR-containing adaptors and thereby inhibits the activation of NF-κB and IRF3 (ref. 78). Another example is the NS3-4A protease of hepatitis C virus, which blocks dsRNA-induced interferon production by interfering with IRF3 phosphorylation[79,80]. NS3-4A cleaves the C-terminal region of Cardif, causing disruption of NF-κB and IRF3 activation, probably by causing mislocalization of cleaved Cardif from mitochondria[76]. NS3-4A also mediates TRIF proteolysis, suggesting multiple functions for this protease[81,82]. Also, the V proteins of paramyxoviruses associate with Mda5 and block 'downstream' signaling pathways[68], and the Kaposi sarcoma–associated herpesvirus immediate-early nuclear transcription factor RTA is a ubiquitin E3 ligase, which can promote IRF7 ubiquitination and degradation in a proteasome-dependent way[83]. Thus, many viral proteins block both TLR- and RIG-I–Mda5–dependent signaling pathways to antagonize type I interferon induction.

Conclusion and future perspectives

Two different classes of PRRs, including multiple TLRs and several RNA helicases, are responsible for the recognition of various viral PAMPs in different cellular compartments and induce antiviral responses via different signaling pathways. However, signaling through these PRRs converges on type I interferon induction and leads to the elimination of viruses. Viruses enter cells by endocytosis or fusion at the plasma membrane, followed by entry to the cytoplasm, where they produce dsRNA and initiate replication. In the cytoplasm, RIG-I and Mda5 are critically involved in the recognition of dsRNA, and the adaptor IPS-1 interacts with RIG-I and Mda5 to facilitate TBK1- and IKKi-mediated IRF3 and IRF7 activation. Thus, these helicases function as cytoplasmic PRRs that are involved in the elimination of actively replicating viruses. TLR3 can also respond to dsRNA. Unlike RIG-I and Mda5, however, TLR3 is localized in endosome-like vesicles and is preferentially expressed in phagocytic cells such as $CD8^+$ DCs. Thus it is possible that after DCs take up apoptotic bodies of virus-infected cells, dsRNAs are delivered into vesicles, where they are uncoated, leading to recognition of their nucleic acids by TLR3.

The finding that TLR3 is also expressed on the cell surface of macrophages and fibroblast cells indicates that TLR3 also recognizes extracellular dsRNA released after lysis of cells in later stages of viral infection. In addition, recognition of viral ssRNA and DNA by endosomally located TLR7-TLR8 and TLR9 in pDCs activates the MyD88-IRAK1-IRF7 pathway and leads to the induction of type I interferon production. It is becoming increasingly apparent that host cells have multiple defensive mechanisms that can eliminate viruses through recognition of various viral PAMPs. Conversely, viruses have developed immune evasion mechanisms specifically targeting those pathways. Further understanding of the mechanisms of virus recognition by those receptors and the mechanisms by which those pathways are controlled will probably result in therapeutically useful tools in the fight against infectious diseases caused by many viruses.

Type I interferon response and DC activation are induced when cells are exposed to DNA released by host damaged cells as well as pathogens. Aberrant induction of type I interferon is known to correlate with the pathogenesis of autoimmune diseases. For example, activation of DCs by chromatin–DNA–containing immunoglobulin G complexes is involved in the pathogenesis of systemic lupus erythematosus[2]. Whereas these complexes are collaboratively recognized by TLR9 and Fc receptors in pDCs, they also stimulate type I interferon induction by cDCs in a TLR-independent way[84,85]. Mice deficient in DNase II, a lysosomal DNase, accumulate undigested DNA in macrophages[86]. Such mice aberrantly produce IFN-β and interferon–inducible genes, which contributes to lethal anemia. However, the lethal anemia is prevented if the mice are crossed with type I interferon receptor–deficient mice but not when they are crossed with TLR9–deficient mice[86], suggesting the possibility that undigested DNA stimulates a type I interferon response via a TLR9–independent mechanism. These observations suggest that an as-yet-unknown protein is responsible for detection of dsDNA in cytoplasm. Synthetic double-stranded B–form DNA or genomic DNA purified from bacteria, viruses or host cells activates DCs (including cDCs and pDCs) and fibroblasts when administered into cytosol, allowing cells to produce type I interferon and chemokines[87]. Such

induction occurs independently of TLRs and RIG-I–Mda5 but is entirely dependent on TBK1 and IKKi[87]. Notably, IPS-1 is probably used in this pathway, as small interfering RNA for IPS-1 abrogates dsDNA-mediated activation of IRF3 and NF-κB[87]. Furthermore, reports have shown that IRF3 is activated by DNA-damaging reagents and that fibroblasts lacking RIP1 are unable to activate NF-κB in response to such reagents[88,89]. Thus, the dsDNA sensor probably uses signaling machinery similar to that in the RIG-I- and Mda5-mediated pathway (**Fig. 3**). The identification of a receptor for DNA detection may not only shed light on the mechanism of DNA virus recognition in the cytoplasm and the pathogenesis of autoimmune diseases and tumors but also assist in the development of powerful adjuvants or DNA vaccines for the treatment of infectious diseases.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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