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The role of viral nucleic acid recognition in dendritic cells for innate and adaptive antiviral immunity

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Abstract

Dendritic cells which are located at the interface of innate and adaptive immunity are targets for infection by many different DNA and RNA viruses. Dendritic cell subpopulations express specific nucleic acid recognition receptors belonging to the Toll-like receptor family (TLR3, 7, 8, 9) and the cytosolic RNA helicase family (RIG-I, MDA5, LGP2). Activation of dendritic cells by viral DNA and RNA via these receptors is essential for triggering the innate antiviral immune response and shaping the ensuing adaptive antiviral immunity. This review will summarize our current knowledge of viral nucleic acid recognition and signaling by Toll-like receptors and RNA helicases focusing on recent evidence for their specific functions in antiviral defense *in vivo*.

Keywords: Dendritic cells; Toll-like receptors; RNA helicases; Virus; Type I interferon

Activation of dendritic cell subpopulations in viral infection

Dendritic cells (DCs), which are present in the tissues of all organs as well as in the circulation and in lymphatic organs, are targets for infection by many different viruses. Viruses entering the body through epithelial surfaces or directly via the blood stream encounter DCs at early time points during infection. DCs are unique in their capacity to migrate from the periphery to lymphoid organs or from the blood stream into lymphatic and peripheral tissues. Virus-infected DCs can therefore transport viruses to other parts of the organism and thus contribute to the spreading of the pathogens to many organs during systemic infection. However, DCs are also equipped with a range of receptors (for example Toll-like receptors, TLRs) that recognize conserved molecular patterns of viruses – either viral proteins or viral nucleic acids with specific distinguishing features (Lund et al., 2003; Krug et al., 2004a, b; Schulz et al., 2005). Triggering of these pattern recognition receptors in virus-infected DCs initiates the innate antiviral immune response, which is essential for limiting further viral dissemination (Dalod et al., 2003; Krug et al., 2004a). The DCs themselves produce inflammatory cytokines and antiviral interferons in response to viral infection. In addition DCs express ligands for activating receptors on the surface of natural killer (NK) cells leading to activation of this innate antiviral effector cell type (Andoniou et al., 2005). DCs are experts in the processing and presentation of viral antigens on MHC class I as well as crosspresentation on MHC class II. The antigen presenting function of DCs is critically involved in the generation of efficient adaptive

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immunity against viruses (Belz et al., 2004). DC activation by viruses via pattern recognition receptors provides the 2nd and 3rd signals required for the priming of naïve-specific T lymphocytes and their differentiation into effector T cells.

DC subpopulations fulfill specific tasks in the generation of antiviral immune responses. Plasmacytoid DCs (pDC), which have also been described as natural interferon producing cells (Perussia et al., 1985; Ronnblom et al., 1983) represent a specialized subset of DCs characterized by their ability to express large amounts of type I interferons and interferon-induced genes in response to viruses as well as synthetic TLR7 and TLR9 ligands (Cella et al., 1999; Krug et al., 2004a, 2001; Siegal et al., 1999).

In response to many viruses pDCs produce 10- to 100-fold more type I interferon on a single cell basis than other cell types such as conventional DC or macrophages (Colonna et al., 2002). In many viral infections pDCs have been shown to release the first wave of type I interferons and thus support subsequent steps of antiviral immunity, including NK cell activation, Th1 cell and CTL differentiation (Dalod et al., 2002, 2003; Krug et al., 2004a; Cervantes-Barragan et al., 2006). It has been shown *in vivo* and *in vitro* however, that cells other than pDCs (for example conventional DCs, macrophages and non-immune cells), also significantly contribute to type I IFN responses during viral infection despite the lower amount of type I interferon produced per cell (Diebold et al., 2003; Barchet et al. 2005; Krug et al., 2004a).

In contrast to other DC populations, pDCs are less efficient in presenting antigens. Several studies report that pDCs are capable of presenting antigens to T cells and triggering T cell proliferation and differentiation to effector cells (Boonstra et al., 2003; Dalod et al., 2003; Salio et al., 2004; Schlecht et al., 2004). However, initial priming of T cell responses in many viral infections relies on conventional DCs, especially the $CD8\alpha^+$ DCs in the murine system (Belz et al., 2004). In addition it has been reported recently that pDCs activated via TLRs may also induce regulatory T cells (Moseman et al., 2004), which may inhibit the expansion and differentiation of antiviral T lymphocytes to prevent overstimulation of the immune system.

What is the molecular basis for the rapid and robust interferon production in pDCs? pDCs recognize viruses via TLRs, particularly via the endosomally located TLR7, TLR8 and TLR9. Downstream signaling of TLR7/8 and TLR9 leads to the activation of IRF7, which is the central transcription factor for expression of type I interferon and interferon inducible genes (Honda et al., 2005b). In contrast to most other cell types pDCs show constitutive expression of IRF7 which is required for the rapid and potent production of the full range of type I IFNs (Izaguirre et al., 2003; Kerkmann et al., 2003). pDC are unique in that IFN-α expression is induced by ligands of TLR7/8 and TLR9 via a supramolecular complex formed between MyD88, TRAF6, IRAK1/4 and IRF7, which leads to direct activation of the constitutively expressed IRF7 only in this specialized cell type (Honda et al., 2004; Kawai et al., 2004).

The cytoplasmatic RNA helicases RIG-I or MDA5 do not contribute to viral recognition in pDCs, because the response of pDCs from mice deficient in RIG-I or the essential signaling adaptor of RIG-I and MDA5 to RNA viruses is comparable to that of wild-type mice (Kato et al., 2005). It has been demonstrated in several studies that pDCs recognize herpes simplex virus type 1 and 2 (HSV-1 and -2) (Krug et al., 2004b; Lund et al., 2003), murine cytomegalovirus (MCMV) (Krug et al., 2004a) and also recombinant replication-deficient adenovirus (Basner-Tschakarjan et al., 2006), via TLR9, whereas Influenza virus (Barchet et al., 2005; Diebold et al., 2004), vesicular stomatitis virus (VSV), Newcastle disease virus (NDV) and Sendai virus (SeV) (Lund et al., 2004) are recognized by TLR7 in pDCs.

Many viruses that enter pDCs and trigger TLRs are prevented from expressing viral genes and replicating their genome. Thus, the activation of the type I IFN response in pDCs most often is not counteracted by viral immune evasion strategies. Accordingly, active viral replication is not required for the induction of type I IFN production in pDCs for many enveloped viruses, for example Influenza virus or herpes viruses (Krug et al., 2004b; Lund et al., 2003). Following endocytic uptake it is assumed that these viruses are retained in acidified endosomal compartments, where TLR7 and TLR9 are localized, so that the viral particles are degraded and viral nucleic acids come into contact with TLR7 and TLR9. A recent report, however, clearly demonstrates that cytosolic viral replication and a transport mechanism involving autophagy is necessary for triggering the TLR7-dependent response to other RNA viruses, such as VSV or SeV in pDCs (Lee et al., 2007). After infection of pDCs with these RNA viruses, viral replication intermediates in the cytosol are internalized in autophagic vesicles which are then directed towards the TLR7-containing lysosomes, where recognition of the viral RNA occurs (Lee et al., 2007).

Toll-like receptors recognize viral DNA and RNA

The Toll-like receptors are a family of type I transmembrane glycoproteins characterized by the extracellular leucine-rich-repeat domain and the cytoplasmatic TIR domain for downstream signaling, which is homologous to the TIR domain of the IL-1 and IL-18 receptors. TLR expression is predominantly found in

various immune cells like dendritic cells, macrophages, B cells and some types of T cells. Moreover TLRs can be expressed by non-immune cells such as fibroblasts and epithelial cells. Among the TLR family a subgroup of mainly intracellularly localized TLRs (TLR3, 7, 8, 9) can be differentiated from TLRs which are expressed on the cell surface (TLR1, 2, 4, 5, 6, 5). Whereas surface-expressed TLRs are mainly involved in the recognition of bacterial and fungal cell wall components as well as some viral proteins, the intracellular/endosomal TLRs have the capacity to detect microbial nucleic acids, particularly viral DNA and RNA. Apart from activating the NFκB and MAPK signaling pathways leading to inflammatory cytokine and chemokine production as well as costimulatory molecule expression, the intracellularly localized nucleic acid recognition receptors TLR3, 7, 8 and 9 specifically trigger type I interferon production via MyD88- and TRIF-dependent signaling pathways.

Toll-like receptor 3

TLR3 binds double-stranded (ds) RNA which is found in dsRNA viruses, such as reovirus, or is generated during replication of single-stranded (ss) RNA viruses such as West Nile virus (Wang et al., 2004) or respiratory syncytial virus (Rudd et al., 2006) or as a by-product of symmetrical transcription of viral DNA, for example from herpes viruses. Another ligand for TLR3 is poly(I:C) which is a synthetic dsRNA mimicking viral infection (Alexopoulou et al., 2001). Studies in TLR3$^{-/-}$ mice identified poly(I:C) and dsRNA as ligands for TLR3, which induce type I IFN and proinflammatory cytokine production. Up-regulation of TLR3 expression by type I IFN amplifies the response to TLR3 ligands.

TLR3 is expressed in conventional DC (cDCs) subpopulations and macrophages as well as non-immune cells, such as fibroblasts and epithelial cells, and the cellular localization of TLR3 varies between different cell types. In conventional DCs TLR3 is thought to be localized in intracellular vesicular structures (Matsumoto et al., 2003). High expression of TLR3 is seen in the subset of CD8α^+CD4$^-$ DC which phagocytose apoptotic cells, including dying cells that are infected by RNA viruses (Edwards et al., 2003).

In other cell types such as fibroblasts or epithelial cells TLR3 is expressed on the cell surface (Matsumoto et al., 2003). Epithelial expression of TLR3 can be found in many different organs including the airways as well as the gastrointestinal and urogenital system. Furthermore, strong expression of TLR3 is detectable in the brain suggesting a specific role in response to viral infections of the central nervous system. Despite this wide expression pattern TLR3 does not appear to be essential for the initial antiviral immune response in several mouse models of viral infection (Edelmann et al., 2004; Lopez et al., 2004).

Downstream signaling of TLR3 is unique among all TLRs, because it is entirely MyD88-independent and is mediated by the cytosolic TIR-domain containing adaptor protein TRIF. TRIF is recruited to the cytoplasmatic TIR domain of TLR3 and interacts with a set of different signaling molecules and kinases which in turn initiate activation of NFκB or IRF3 and IRF7 leading to type I interferon induction. Interaction of TRIF with TRAF3 allows complex formation with the non-canonical IKKs-TBK1 and IKKε leading to the activation of IRF3 and IRF7, which form homo- or heterodimers upon phosphorylation and are then translocated to the nucleus to induce type I IFN and IFN inducible gene expression (Fitzgerald et al., 2003; Sharma et al., 2003; Yamamoto et al., 2003). NFκB activation by TLR3 ligands is mediated in two ways – via association of TRIF with RIP1 or via interaction of TRIF with TRAF6, which in turn activates TAK1. Both RIP1 and TAK1 mediate activation of canonical IKKs (IKKα, IKKβ) resulting in IκB degradation and NFκB translocation to the nucleus (Meylan et al., 2004; Sato et al., 2007).

The role of TLR3 in antiviral immunity remains unclear so far. Most studies did not find an essential role of TLR3 for the generation of effective antiviral immune responses. TLR3-deficient mice are as susceptible to reovirus, VSV and LCMV infection as wild-type mice and there was no significant difference in generating specific CD4 and CD8 T cell responses to these viruses (Edelmann et al., 2004). Interestingly, however, virally induced CNS injury was improved by TLR3-deficiency suggesting that inflammatory responses mediated by TLR3 are at least partially responsible for a breakdown of the blood–brain barrier which facilitates and enhances virus entry into the brain. TLR3-deficient mice survived viral CNS infection with West Nile virus (Wang et al., 2004), which is characterized by meningitis and encephalitis induced by inflammatory mediators, for longer time periods than wild-type mice. Similarly, in murine influenza A virus infection TLR3-mediated inflammatory responses in the lung contribute significantly to host morbidity and lethality. Despite higher pulmonary viral loads TLR3$^{-/-}$ mice showed less inflammation and better survival (Le Goffic et al., 2006). Interestingly, human lung epithelial cells express proinflammatory cytokines including IL-6 and IL-8 upon infection with influenza A virus in a TLR3-dependent manner (Le Goffic et al., 2007), suggesting that TLR3-mediated inflammatory responses may also contribute to influenza virus-induced lung pathology in humans.

The role of TLR3 in MCMV infection is more controversial. Whereas Edelmann et al. (2004) claimed

no impairment in antiviral response to MCMV in TLR3$^{-/-}$ mice, another publication suggested a higher susceptibility to MCMV in the absence TLR3 (Tabeta et al., 2004). In this study TLR3 deficiency results in higher splenic viral titers compared to wild-type mice. Similar observations were made when infecting TRIF$^{Lps2/Lps2}$ mice with MCMV (Hoebe et al., 2003). The increased viral load was accompanied by a decreased cytokine response which mostly affected type I IFN and to a lesser extent IL12p40 and IFN-γ produced by NK and NKT cells (Tabeta et al., 2004).

Crosspriming occurs most efficiently in the specialized subpopulation of CD8α^+ DCs, which have a high capacity for internalization of apoptotic cells. TLR3 which is expressed at high levels in CD8α^+ DCs (Edwards et al., 2003) plays a critical role for cross-priming of CTLs directed against viruses that do not infect DCs directly. CD8α^+ DCs are activated by viral RNA contained in internalized virally infected apoptotic cells and crosspresent viral antigens to specific CD8$^+$ T cells in a TLR3-dependent manner (Schulz et al., 2005).

Toll-like receptor 9

TLR9 has been described to recognize bacterial DNA or synthetic oligodesoxyribonucleotides (ODN) containing specific unmethylated CpG sequence motifs. These can also be found in the genome of DNA viruses. Viruses recognized by TLR9 are HSV type 1 and 2 (Hochrein et al., 2004; Krug et al., 2004b), MCMV (Krug et al., 2004a; Lund et al., 2003; Tabeta et al., 2004), adenovirus (Basner-Tschakarjan et al., 2006) and baculovirus (Abe et al., 2005). Recognition of HSV or MCMV by TLR9 of pDCs results in robust induction of type I interferon and inflammatory cytokines (Krug et al., 2004a, b; Lund et al., 2003). CpG ODN class A, B and C have been designed to trigger primarily type I IFN response (A) or costimulatory molecule expression and inflammatory cytokine production (B) or both (C) in pDCs. CpG-B and CpG-C additionally trigger B cell stimulation via TLR9 (Krug et al., 2001; Verthelyi et al., 2001; Hartmann et al., 2003).

Induction of type I IFNs by TLR9 ligands in pDCs depends on retention of the ligand–receptor complex within early endosomes at a pH value between 6.2 and 5.5 (Guiducci et al., 2006). Delivery of TLR9 ligands to late endosome with lower pH values (<4.5) impairs the induction of type I IFNs and promotes inflammatory cytokine and costimulatory molecule expression in pDCs (Guiducci et al., 2006). Early endosomal retention is achieved by using CpG-A, which forms aggregates or by transfecting CpG-B with cationic liposomes or delivering CpG DNA in the form of immune complexes (Kerkmann et al., 2005; Means et al., 2005; Guiducci et al., 2006). TLR9 ligands delivered within herpes virus particles also seem to be retained long enough in the early endosomal compartment to trigger type I IFN responses efficiently.

Upon ligand binding the TIR domain of TLR9 recruits MyD88 which forms a supramolecular complex with TRAF6, IRAK1, IRAK4 and IRF7 (Honda et al., 2004; Kawai et al., 2004). IRF7 becomes activated upon phosphorylation which results in homodimerization of IRF7 or heterodimerization of IRF3 and IRF7. These dimers then translocate to the nucleus and induce expression of type I interferon and interferon inducible genes. Several proteins were implicated in the phosphorylation of IRF7 including IRAK-1, IKKα, and a precursor of osteopontin (Hoshino et al., 2006; Shinohara et al., 2006; Uematsu et al., 2005). Lack of IRAK1 abolishes IFN-α production and IRF7 activation in response to TLR7, TLR8 and TLR9 ligands (Uematsu et al., 2005).

IRF7 plays the role of a "master regulator" in the induction of type I interferon and IFN inducible genes in response to viruses (Honda et al., 2005a). The currently accepted two step model of positive feedback regulation of type I interferon gene expression consists of an initial phase with activation of IRF7 expressed constitutively at low levels, formation of IRF7-homodimers or IRF7/IRF3-heterodimers and induction of small amounts of IFN-β/IFN-α and chemokines. During the second phase secreted type I IFNs signal via type I IFN receptor in an autocrine and paracrine manner. Downstream signaling of the type I IFN receptor induces a strong up-regulation of IRF7 production leading to full expression of type I IFN genes (positive feedback loop). Although pDCs constitutively express IRF7 at higher levels than other cells, they also depend on further up-regulation of IRF7 by type I IFN signaling to be able to sustain high level production of type I IFNs (Honda and Taniguchi, 2006; Honda et al., 2005b).

In contrast to pDCs, type I IFN production in myeloid DCs in response to DNA viruses is mediated by both TLR9-dependent and TLR9/MyD88-independent pathways. Downstream signaling of TLR9 in murine myeloid DCs shows differences compared to pDCs. In myeloid DCs IRF1 plays a crucial role in the downstream signaling of TLR9. IRF1$^{-/-}$ mice show an impaired induction of IFN-β, iNOS and IL-12p35 upon stimulation with TLR9 ligand CpG-B, whereas type I interferon response of pDCs is not affected by lack of IRF1 (Negishi et al., 2006; Schmitz et al., 2007).

What is the role of TLR9 for antiviral immunity *in vivo*? Several studies have described increased susceptibility of TLR9$^{-/-}$ mice to systemic murine cytomegalovirus (MCMV) infection (Tabeta et al. 2004; Krug et al., 2004a; Delale et al., 2005). We could show that TLR9-dependent recognition of MCMV by pDCs and conventional DC is clearly involved in the innate

immune response to MCMV. In the very early phase of systemic MCMV infection pDCs and conventional DCs release the first wave of type I interferons and inflammatory cytokines including IL-12. The expression of these cytokines peaks between 36 and 38 h post-infection (Dalod et al., 2002; Orange and Biron, 1996b) and triggers non-specific NK cell activation at this time point. Control of viral replication and clearance of the virus from infected organs during the first week of MCMV infection relies mostly on NK cells, which produce IFN-γ (mediated by IL-12 and IL-18) and kill infected cells (Orange and Biron, 1996a). We found a significant reduction in IFN-α and IL-12 serum levels in TLR9- and MyD88-deficient mice at 36 h after infection. However, IFN-α serum levels comparable to those of wild-type mice could be observed in TLR9- and MyD88-deficient mice at later time points suggesting the existence of an additional TLR9/MyD88-independent type I IFN induction mechanism with delayed kinetics. Loss of the early IFN-α peak is due to an impaired function of pDCs in TLR9$^{-/-}$ mice as MCMV recognition and type I IFN production are TLR9-dependent in this cell type. pDC depletion *in vivo* led to markedly reduced IFN-α levels at 36 h after infection reflecting their almost exclusive role for IFN-α production at this early time point. In contrast to IFN-α, IL-12 production was severely impaired in TLR9- and MyD88-deficient mice at all time points reflecting the requirement of TLR9 for IL-12 responses to MCMV in all DC subpopulations. IFN-γ production by NK cells which is induced by IL-12 and IL-18 was also significantly reduced in the absence of TLR9 or MyD88. These defects in the innate immune response to MCMV correlated with higher viral titers in spleen and liver of TLR9$^{-/-}$ and MyD88$^{-/-}$ mice on the C57BL/6 background reflecting increased susceptibility to MCMV in the early phase of the infection (Delale et al., 2005; Krug et al., 2004a).

A recent report showed that pDCs which are recruited to the vaginal mucosa after local infection with HSV-2 are activated to produce type I IFN in a TLR9-dependent manner, thus reducing local viral replication and pathology (Lund et al., 2006). In two mouse models of local infection with HSV-1 (footpad or eye infection), however, we did not find a significant difference in viral titers between TLR9$^{-/-}$ and MyD88$^{-/-}$ mice and wild-type mice (Krug et al., 2004b). The requirement for TLR9 in the innate immune response to herpes viruses *in vivo* is influenced by the site of virus entry and the recruitment of DC subpopulations to the infected tissue. All of the described *in vivo* studies show that in addition to TLR9 other pattern recognition receptors and signaling pathways responding to herpes virus infection must exist, which can partially compensate for the lack of TLR9 and other MyD88-dependent receptors (Krug et al., 2004b; Hochrein et al., 2004; Lund et al., 2006). The cytosolic DNA receptor which has been described recently (Stetson and Medzhitov, 2006; Ishii et al., 2006; Takaoka et al., 2007) may also be involved in the TLR9-independent component of the immune response to herpes virus infections.

Toll-like receptor 7 and 8

TLR7 and TLR8 are both located on the X chromosome and are homologous to each other. GU-rich ssRNA sequences of viral or host origin, poly-U RNA and specific siRNA sequences are ligands for both receptors, whereas synthetic imidazoquinoline derivatives have been designed to specifically activate TLR7 or TLR8 or both receptors (Heil et al., 2004; Hemmi et al., 2002; Diebold et al., 2004; Jurk et al., 2002; Hornung et al., 2005; Gorden et al., 2005). TLR7 is expressed in human and murine pDCs, conventional DCs and B cells, whereas TLR8 seems to be functional mainly in human monocytes and myeloid DCs. The role of TLR8 in the murine immune system remains to be elucidated. Similar to what has been described for TLR9, activation of TLR7 and TLR8 by specific ligands occurs in the acidified endosomal compartment. The MyD88-dependent signal transduction pathway downstream of TLR7 and 8 is very similar to TLR9-mediated signaling.

Several viruses are recognized by pDCs in a TLR7-dependent manner including influenza virus (Diebold et al., 2004; Barchet et al., 2005), Newcastle disease virus (NDV) (Kato et al., 2005), vesicular stomatitis virus (VSV) (Lund et al., 2004), coronaviruses (Cervantes-Barragan et al., 2006) and RNA viruses (Heil et al., 2004; Beignon et al., 2005). TLR7 does not seem to play an essential role in the innate immune response to RNA virus infection *in vivo*. We could not find a significant difference between MyD88$^{-/-}$ and wild-type mice in the susceptibility to intranasal influenza virus infection and type I IFN response to intravenously injected influenza virus was only partially reduced in the MyD88-deficient animals (Barchet et al., 2005). At high viral doses type I IFN response to systemic VSV infection is TLR7-dependent and mediated by pDCs, whereas at lower doses TLR7-independent type I IFN induction pathways play the major role. A recent study demonstrates an essential function of TLR7 for the innate immune response to systemic coronavirus infection in mice (Cervantes-Barragan et al., 2006). pDCs, but not conventional DCs are capable of producing significant amounts of type I IFN in response to the rapidly replicating coronaviruses and this response is entirely TLR7-dependent. pDC depletion led to abrogation of type I IFN response and increased disease severity (Cervantes-Barragan et al., 2006). It can be concluded that TLR7 (and possibly TLR8 in the human system)

contribute to the initiation of the antiviral immune response against viruses which specifically target pDCs. In addition the ubiquitously expressed RNA helicases provide protection against RNA viruses in all cell types (see below). Depending on the route and kinetics of infection, cellular tropism, viral entry and replication as well as immune evasion mechanisms of individual virus strains one or the other viral RNA recognition pathway may dominate the innate antiviral immune response *in vivo*.

What is the role of TLRs for antiviral immune responses in humans?

As suggested by the murine *in vivo* studies there is considerable redundancy in TLR-dependent and TLR-independent viral pattern recognition mechanisms also in the human system. IRAK-4-deficient patients are prone to invasive infection with pneumococci, but are resistant to natural viral infection (Picard et al., 2003). IRAK4-deficient PBMC do not respond to any of the MyD88-dependent TLR ligands, but are capable of type I IFN production in response to many DNA and RNA viruses. There also seems to be considerable redundancy between different TLRs, because in contrast to the IRAK4-deficient patients, which show no significant defects in antiviral responses (Picard et al., 2003), individuals with autosomal recessive deficiency in the intracellular protein Unc-93b have an impaired antiviral type I IFN response and are susceptible to HSV-1 encephalitis (Casrouge et al., 2006). Unc-93b-deficiency prevents signaling in response to ligands of the endosomally localized TLR3, TLR7, TLR8 and TLR9 (Tabeta et al., 2006). In addition, the Unc-93b protein, which is localized in the endoplasmic reticulum, is required for efficient crosspresentation and MHC class II presentation of exogenous antigens (Tabeta et al., 2006). Therefore, Unc-93b-deficiency may affect innate and adaptive antiviral immune responses at the same time. No human deficiencies or mutation in the RIG-I/MDA5 pathway of RNA virus recognition have been described so far. However, the critical role of this major pathway for innate antiviral immune responses in humans is greatly supported by the existence of viral immune evasion strategies, which, for example, are employed by hepatitis C virus and paramyxoviruses and are directed against several signaling molecules in this pathway (see below).

RNA helicases sense intracellular viral RNA

In addition to the TLRs, a new family of viral pattern recognition receptors consisting of RNA helicases retinoic acid inducible gene I (RIG-I), melanoma differentiation antigen 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2) – was discovered and characterized in the last 3 years. These molecules, which are localized in the cytosol, bind specific RNA molecules derived from the genome of different RNA viruses and, with the exception of LGP2 which does not signal itself, trigger a signaling cascade leading to the production of type I IFNs and of proinflammatory cytokines in response to viral infection.

RIG-I

Retinoic-acid-inducible protein I (RIG-I) is a DExD/H box-containing RNA helicase that was originally identified as an enhancer of type I IFN expression in response to dsRNA poly (I:C) (Yoneyama et al., 2004). DExD/H box RNA helicases are defined by their ability to unwind dsRNA using their intrinsic ATPase activity. In addition to the C-terminal helicase domain RIG-I contains two caspase recruitment domains (CARD) at its N terminus (Zhang et al., 2000). CARDs are found in a number of caspases, but also in other proteins such as NOD1 and NOD2, involved in sensing intracellular bacterial products (Inohara and Nunez, 2003). The functions of RIG-I have been analyzed in detail in the first publication by Yoneyama et al. (2004) using deletion constructs of full length RIG-I. Overexpression of the N-terminal region of RIG-I (delta RIG-I) comprising the two tandem CARDs is sufficient to induce IRF3 and NFκB activation even in the absence of a dsRNA stimulus or viral challenge. The mutant of RIG-I lacking the CARD domain is not capable of transmitting signals. This N-terminally truncated molecule even has a dominant negative effect since expression of this domain alone prevents activation of IRF3 by dsRNA transfection or NDV infection (Yoneyama et al., 2004).

Since CARDs are known to engage in homophilic protein–protein interactions in other signal transduction pathways, it was therefore likely that the CARDs of RIG-I interact with other CARD containing molecules to activate downstream signaling molecules. The adaptor protein that links RIG-I to the activation of TBK1/IKKε and IKKβ was identified and functionally characterized by four independent groups and 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), respectively. IPS-1 contains an N-terminal CARD domain that interacts with the tandem CARD domains of RIG-I and a C-terminal hydrophobic transmembrane (TM) domain that localizes it to the outer mitochondrial membrane (Seth et al., 2005). Deletion analyses have shown that both the CARD and the transmembrane

domain are essential for the function of IPS-1. The mitochondrial localization of IPS-1 is essential for its activity because deletion of the TM domain, which leads to cytosolic expression of the protein, abolishes the signaling function of IPS-1. The binding of dsRNA to the helicase domain of RIG-I likely induces a conformational change that exposes the N-terminal CARD domains to recruit its signaling adaptor IPS-1. This interaction subsequently induces IFN-α/INF-β and IFN-induced antiviral effector mechanisms that suppress virus replication (Meylan et al., 2006).

In a recent publication Saito et al. reported that an internal repressor (or regulatory) domain (RD) at the C-terminus controls RIG-I multimerization and IPS-1 interaction during virus infection and RNA binding (Saito et al., 2007). Expression of the C-terminal RD domain, encompassing the amino acids 735–925 of the RIG-I protein, prevents signaling to the IFN-β promoter and increased cellular permissiveness to hepatitis C virus, whereas deletion of the RIG-I RD results in constitutive signaling. Saito et al. suggest a model of RIG-I autoregulation and signaling predicting that in resting cells the C-terminal RD mediates a conformation of RIG-I that masks the CARDs from signaling. Once the cell is infected with virus, RIG-I activation and signaling occurs in a stepwise manner involving dsRNA binding and conformational changes that subsequently facilitate self-association and interaction with the IPS-1 signaling adaptor. These conformational changes comprising displacement of the RD and unmasking of the CARDs for signaling via IPS-1 might be triggered by ATP hydrolysis. Saito et al. also identified an analogous RD within the C terminus of LGP2 suggesting that LGP2 might inhibit RIG-I through their RD interactions. LGP2 is a close relative of RIG-I, which lacks the CARDs, but is capable of binding dsRNA (Yoneyama et al., 2005). Thus, LGP2 may act as a postinduction repressor of RIG-I signaling (Rothenfusser et al., 2005).

From several studies it is known that RIG-I is essential for antiviral responses to a specific set of RNA viruses belonging to *Flaviviridae*, *Paramyxoviridae*, *Orthomyxoviridae* and *Rhabdoviridae* (Kato et al., 2006; Sumpter et al., 2005; Yoneyama et al., 2004). Since RNA is a fundamental entity of most living organisms and is found in abundance in host cells, a molecular pattern must exist that enables RIG-I to discriminate between viral RNA and host RNA species in the cytoplasm of infected cells to prevent constant induction of type I IFNs which would lead to autoimmune responses. Two recent studies have identified features of viral RNA that are structurally different to host RNA. Hornung et al. (2006) demonstrated that ssRNA synthesized *in vitro* acquires a 5′-triphosphate moiety which is crucial for IFN production in host cells. Furthermore, they demonstrated that RNA isolated from Rabies virus (RV) infected cells is a potent IFN inducer, whereas RNA from non-infected cells and dephosphorylated RNA isolates abrogated this IFN response. The 5′-phosphorylation status and the absence of a 7-methyl-guanosine cap provided by the viral polymerase is critical for recognition by RIG-I. Taken together, the results of Hornung et al. (2006) show that RIG-I directly recognizes 5′-triphosphate single stranded or double stranded RNA independently of viral replication. In the second study, Pichlmair et al. (2006) found that Influenza A virus, which is known to be sensed by both RIG-I and TLR3, did not generate dsRNA upon infection of bone-marrow-derived dendritic cells. Moreover, they observed that Influenza virus ssRNA, which is uncapped and bears 5′-triphosphates, associated with and activated RIG-I.

IPS-1 functions as critical link between viral detection by RIG-I and the downstream signaling events leading to interferon production. This receptor–adapter interaction results in the activation of the noncanonical kinases TBK1/IKKε, which in turn induces dimerization of phosphorylated IRF3 and IRF7 and translocation to the nucleus where they activate the transcription of type I IFN genes (Kawai et al., 2005). Coimmunoprecipitation experiments suggest that IPS-1 interacts with TBK1 and recruits endogenous IRF3 in a virus-inducible manner (Xu et al., 2005). Recently, TRAF3 was shown to be critically involved in IPS-1-mediated IFN-α production and antiviral responses through a direct interaction between the TRAF domain of TRAF3 and a TRAF interaction motif within IPS-1 (Saha et al., 2006). There is evidence that the TBK1/IKKε adaptor protein TANK plays a role in IPS-1-TRAF3-mediated activation of TBK1/IKKε (Guo and Cheng, 2007). RIP1 and FADD are additional molecules that have been reported to be required for type I IFN production in response to dsRNA (Balachandran et al., 2004).

Another branch of IPS-1 signaling leads to the activation of the IKK complex resulting in activation of NFκB that controls the expression of genes encoding inflammatory responses, but also expression of IFN-β. Previous work showed that IPS-1 activates a NFκB-dependent reporter construct in cultured cells (Matsuda et al., 2003). One of the key signaling proteins in the NFκB pathway is TRAF6, an essential upstream regulator of the IKK complex. IPS-1 binds to TRAF6 upon overexpression in mammalian cells and in a yeast-two hybrid screen (Seth et al., 2005; Xu et al., 2005). Xu et al. (2005) reported that endogenous IPS-1 also interacts with TRAF6. In addition, they showed that IPS-1 fails to activate NFκB in the absence of TRAF6. Seth et al. (2005) reported that virus-mediated induction of the IFN-β gene is not abolished in TRAF6 deficient cells and that a mutant IPS-1 protein lacking the TRAF6 binding domain is still capable of inducing IFN-β. Thus, TRAF6 seems to be required for NFκB

activation but not IFN-β induction downstream of IPS-1 which is mainly mediated by TBK1/IKKε.

In vitro studies performed with primary cells obtained from RIG-I knockout mice confirmed that RIG-I plays an essential role in eliciting immune responses against specific negative strand and positive strand RNA viruses such as NDV, SeV, VSV, Japanese encephalitis virus (JEV) and Influenza virus in various cell types with the exception of pDCs (Kato et al., 2006). The experiments demonstrated that type I IFN production by RIG-I deficient fibroblasts and conventional DCs is severely impaired in response to these RNA viruses. In contrast, pDCs lacking RIG-I show normal type I IFN responses to NDV or VSV for example, as the pDC response to these viruses is mediated by TLR7 (Kato et al., 2005). The studies performed so far indicate that the RNA helicases RIG-I and MDA5 as well as their common signaling adaptor IPS-1 are dispensible for viral triggering of type I IFN responses in pDCs (Kato et al., 2006).

MDA5

MDA5 is another DExD/H-box-containing RNA helicase that is involved in the sensing of intracellular dsRNA and the induction of type I IFN in response to RNA viruses (Andrejeva et al., 2004). It is the closest relative of RIG-I, exhibiting 23 and 35% amino acid homology in the N-terminal CARD and C-terminal helicase domain, respectively. It was reported in a previous publication, that MDA5, which was then called Helicard, is cleaved by caspases upon induction of apoptosis, thereby separating the CARD domains from the C-terminal helicase domain, which localizes to the nucleus where it is involved in DNA degradation and nuclear remodelling during apoptotic cell death (Kovacsovics et al., 2002). Furthermore, MDA5 has been implicated in the regulation of the growth and differentiation of melanoma cells (Kang et al., 2002). MDA5 is ubiquitously expressed in low abundance and similarly to RIG-I and LGP2 its expression is induced by type I IFN. Like RIG-I, MDA5 interacts with the adapter protein IPS-1 upon ligand binding leading to activation of protein kinases that subsequently activate transcription factors IRF3, IRF7 and NFκB, respectively. It was found recently by Sato et al. (2007) that in contrast to RIG-I, the C-terminal regulatory domain of MDA5 did not exert a repressor function on MDA5 signaling, which was also not inhibited by LGP2. MDA5 overexpression in cell lines even at low levels induces IFN-β promoter activity in the absence of ligand binding. Thus, MDA5 expression which is up-regulated in response to type I IFN signaling may function as an amplifier of type I IFN production even in the absence of specific MDA5 ligands.

Although RIG-I and MDA5 are similar proteins inducing synthesis of type I IFN via the same signaling pathway, they are specialized in the recognition of different viruses. *In vitro* studies performed with embryonic fibroblasts and conventional DCs derived from MDA5 knockout mice have shown that MDA5 is specifically required for the recognition of intracellular poly (I:C) dsRNA (but not *in vitro* transcribed PPP-RNA) and picornaviruses such as encephalomyocarditis virus (EMCV), Theiler's virus and Mengo virus, whereas RIG-I was not necessary for the response to poly (I:C) or picornaviruses (Kato et al., 2006; Gitlin et al., 2006). In addition, it was shown that MDA5 might also play a role in the measles virus (MV) induced activation of IFN-β mRNA synthesis since A549 cells transfected with MDA5 showed a strong activation of the IFN-β promoter upon infection with MV. In contrast, the virus did not enhance reporter gene activity in cells that overexpressed RIG-I (Berghall et al., 2006). MDA5 was not required for recognition of VSV, NDV, JEV, SeV and influenza virus, indicating that RIG-I was the predominant pattern recognition receptor for these viruses. It is currently unknown which specific viral RNA motifs in picornaviruses are recognized by MDA5 and how ligand specificity is determined on the molecular level.

RIG-I and MDA5 are essential mediators of antiviral immunity *in vivo*

The *in vivo* relevance of the RIG-I and MDA5 pathways for innate antiviral immune responses was addressed by the generation of knockout mice. The specific susceptibility of MDA5-deficient mice to encephalomyocarditis virus (EMCV) infection was shown in two studies (Kato et al., 2006; Gitlin et al., 2006). In a direct comparison with IFNAR$^{-/-}$ and MyD88$^{-/-}$ mice Kato et al. (2006) could show that survival upon EMCV infection is as dramatically reduced in MDA5$^{-/-}$ mice as in IFNAR$^{-/-}$ mice. MyD88-deficient mice were only slightly more susceptible to EMCV infection than wild-type mice. Survival after EMCV infection was not affected by RIG-I- or TLR3-deficiency. Studies in the IPS-1 knockout mouse confirmed the dramatic reduction in the innate immune response to EMCV infection, which correlated with increased viral titers and decreased survival, in the absence of the central downstream signaling adaptor of MDA5 and RIG-I (Kumar et al., 2006). Only few *in vivo* experiments with RIG-I-deficient mice have been published so far, because for unknown reasons most of the RIG-I-knockout mice (on a 129/C57BL/6 mixed background) died *in utero* or within a few weeks after birth (Kato et al., 2006). After crossing with ICR mice healthy RIG-I$^{-/-}$ mice were obtained and compared with littermate controls in the

JEV and VSV infection models. RIG-I$^{-/-}$ mice were more susceptible to JEV and VSV infection than wild-type mice and this correlated with reduced type I IFN responses. MyD88-dependent type I IFN production induced by JEV and VSV played only a minor role in these infection models (Kato et al., 2006). In accordance with these results IPS-1-deficient mice succumbed rapidly to VSV infection (Sun et al., 2006). As pointed out by the study of Sun et al. (2006), type I IFN responses to systemic VSV infection were not entirely abrogated in IPS-1-deficient mice, suggesting that TLR7-dependent pDC-mediated type I IFN release at least partially compensates for the dysfunctional RNA helicase pathway, but this compensatory mechanism cannot prevent death after infection with high doses of VSV.

The RNA helicase pathway as target for viral immune evasion strategies

Viruses have adapted strategies to evade or inhibit key elements of antiviral immunity. A number of viral proteins inhibit host innate immune responses, prevent viral antigen presentation and abrogate induction of cell death. Since the cytoplasmic RIG-I/MDA5 system is critical for host defense against RNA viruses, the signaling cascades induced by these sensors are also targeted by viruses. Various proteins encoded by RNA viruses have been shown to antagonize the cytoplasmic RNA helicase pathways.

IFN antagonists of negative strand RNA viruses can interfere with this pathway by hiding their RNA (Influenza A virus NS1 protein) or binding to the dsRNA receptor (Paramyxovirus V proteins) or preventing activation of downstream factors such as IRF3 (Ebola virus VP35, Rhabdovirus P), respectively.

The non-structural protein 1 (NS1) of the Influenza viruses is a dsRNA-binding protein that acts as an IFN antagonist (Garcia-Sastre et al., 1998). By binding to dsRNA NS1 disguises the viral dsRNA pattern from the cytoplasmic receptors and inhibits IFN-α/INF-β induction via IRF3 (Garcia-Sastre, 2004; Talon et al., 2000). RIG-I was recently demonstrated to be essential for the induction of IFN-β by Influenza virus in murine cells. Furthermore, it was observed that NS1 colocalizes with RIG-I (Mibayashi et al., 2007), suggesting that NS1 forms a complex with RIG-I and IPS-1 during viral infection, resulting in inhibition of further downstream signaling. Respiratory syncytial virus (RSV) specifically interferes with IRF3 activation and IFN-β response: Two viral proteins of RSV, NS1 and NS2, are involved in blocking the pathway leading to IRF3 phosphorylation, although the activation of NFκB and AP-1 is unaffected (Bossert et al., 2003). IRF3 phosphorylation by TBK1 was identified as target of the Rabies virus phosphoprotein P (Brzozka et al., 2005). Recently it was shown that the NY-1 Hantavirus G1 cytoplasmic tail inhibits RIG-I- and TBK1-directed interferon responses (Alff et al., 2006).

The V proteins of paramyxoviruses target MDA5, but not RIG-I. The V proteins of this diverse group of viruses bind MDA5 via their highly conserved cysteine-rich C-terminal domain. This suggests that paramyxoviruses use this interaction to reduce the amount of IFN released by infected cells (Andrejeva et al., 2004; Childs et al., 2007). Furthermore, it was shown that MDA5 is also a target of picornaviruses since MDA5 is degraded in poliovirus-infected cells. Interestingly, MDA5 is not directly cleaved by virus-encoded proteinases. Degradation of MDA5 in poliovirus-infected cells occurs in a proteasome- and caspase-dependent manner and correlates with the induction of apoptosis in poliovirus-infected cells. Poliovirus-induced MDA5 cleavage attenuates the production of type I IFN, thereby allowing higher levels of viral replication and dissemination in the host (Barral et al., 2007).

Hepatitis C virus (HCV) encodes the protease NS3/4A which targets IPS-1 by cleaving it at position Cys-508, thereby dislocating it from the mitochondrial membrane and thus abrogating further downstream signaling to type I IFN and NFκB-dependent target gene expression (Li et al., 2005; Meylan et al., 2005). Interestingly, IPS-1 was also found to be localized to the cytosol and not the mitochondria in liver tissue from patients chronically infected with HCV (Loo et al., 2006). Inhibitors of the HCV NS3/4A, which have originally been designed to inhibit HCV replication, are able to prevent IPS-1 cleavage and restore the RIG-I-mediated innate immune response to HCV (Johnson et al., 2007). Therefore, NS3/4A inhibitors which are already being tested in clinical trials may have therapeutic potential for chronic hepatitis C infection. The fact that RNA viruses have developed so many effective strategies to interfere with the RNA helicase pathway of viral recognition during coevolution with their host provides further proof for the central role of this pathway in antiviral immune defense.

References

Abe, T., Hemmi, H., Miyamoto, H., Moriishi, K., Tamura, S., Takaku, H., Akira, S., Matsuura, Y., 2005. Involvement of the Toll-like receptor 9 signaling pathway in the induction of innate immunity by baculovirus. J. Virol. 79, 2847–2858.

Alexopoulou, L., Holt, A.C., Medzhitov, R., Flavell, R.A., 2001. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature 413, 732–738.

Alff, P.J., Gavrilovskaya, I.N., Gorbunova, E., Endriss, K., Chong, Y., Geimonen, E., Sen, N., Reich, N.C., Mackow, E.R., 2006. The pathogenic NY-1 hantavirus G1 cytoplasmic

tail inhibits RIG-I- and TBK-1-directed interferon responses. J. Virol. 80, 9676–9686.

Andoniou, C.E., van Dommelen, S.L., Voigt, V., Andrews, D.M., Brizard, G., Asselin-Paturel, C., Delale, T., Stacey, K.J., Trinchieri, G., Degli-Esposti, M.A., 2005. Interaction between conventional dendritic cells and natural killer cells is integral to the activation of effective antiviral immunity. Nat. Immunol. 10, 1011–1019.

Andrejeva, J., Childs, K.S., Young, D.F., Carlos, T.S., Stock, N., Goodbourn, S., Randall, R.E., 2004. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. Proc. Natl. Acad. Sci. USA 101, 17264–17269.

Balachandran, S., Thomas, E., Barber, G.N., 2004. A FADD-dependent innate immune mechanism in mammalian cells. Nature 432, 401–405.

Barchet, W., Krug, A., Cella, M., Newby, C., Fischer, J.A., Dzionek, A., Pekosz, A., Colonna, M., 2005. Dendritic cells respond to influenza virus through TLR7- and PKR-independent pathways. Eur. J. Immunol. 35, 236–242.

Barral, P.M., Morrison, J.M., Drahos, J., Gupta, P., Sarkar, D., Fisher, P.B., Racaniello, V.R., 2007. MDA-5 is cleaved in poliovirus-infected cells. J. Virol. 81, 3677–3684.

Basner-Tschakarjan, E., Gaffal, E., O'Keeffe, M., Tormo, D., Limmer, A., Wagner, H., Hochrein, H., Tuting, T., 2006. Adenovirus efficiently transduces plasmacytoid dendritic cells resulting in TLR9-dependent maturation and IFN-alpha production. J. Gene Med. 8, 1300–1306.

Beignon, A.S., McKenna, K., Skoberne, M., Manches, O., DaSilva, I., Kavanagh, D.G., Larsson, M., Gorelick, R.J., Lifson, J.D., Bhardwaj, N., 2005. Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor–viral RNA interactions. J. Clin. Invest. 115, 3265–3275.

Belz, G.T., Smith, C.M., Eichner, D., Shortman, K., Karupiah, G., Carbone, F.R., Heath, W.R., 2004. Cutting edge: conventional CD8 alpha+ dendritic cells are generally involved in priming CTL immunity to viruses. J. Immunol. 172, 1996–2000.

Berghall, H., Siren, J., Sarkar, D., Julkunen, I., Fisher, P.B., Vainionpaa, R., Matikainen, S., 2006. The interferon-inducible RNA helicase, mda-5, is involved in measles virus-induced expression of antiviral cytokines. Microb. Infect. 8, 2138–2144.

Boonstra, A., Asselin-Paturel, C., Gilliet, M., Crain, C., Trinchieri, G., Liu, Y.J., O'Garra, A., 2003. Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential toll-like receptor ligation. J. Exp. Med. 197, 101–109.

Bossert, B., Marozin, S., Conzelmann, K.K., 2003. Nonstructural proteins NS1 and NS2 of bovine respiratory syncytial virus block activation of interferon regulatory factor 3. J. Virol. 77, 8661–8668.

Brzozka, K., Finke, S., Conzelmann, K.K., 2005. Identification of the rabies virus alpha/beta interferon antagonist: phosphoprotein P interferes with phosphorylation of interferon regulatory factor 3. J. Virol. 79, 7673–7681.

Casrouge, A., Zhang, S.Y., Eidenschenk, C., Jouanguy, E., Puel, A., Yang, K., Alcais, A., Picard, C., Mahfoufi, N., Nicolas, N., Lorenzo, L., Plancoulaine, S., Senechal, B., Geissmann, F., Tabeta, K., Hoebe, K., Du, X., Miller, R.L., Heron, B., Mignot, C., de Villemeur, T.B., Lebon, P., Dulac, O., Rozenberg, F., Beutler, B., Tardieu, M., Abel, L., Casanova, J.L., 2006. Herpes simplex virus encephalitis in human UNC-93B deficiency. Science 314, 308–312.

Cella, M., Jarrossay, D., Facchetti, F., Alebardi, O., Nakajima, H., Lanzavecchia, A., Colonna, M., 1999. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. Nat. Med. 5, 919–923.

Cervantes-Barragan, L., Zust, R., Weber, F., Spiegel, M., Lang, K.S., Akira, S., Thiel, V., Ludewig, B., 2006. Control of coronavirus infection through plasmacytoid dendritic cell-derived type I interferon. Blood 109, 1131–1137.

Childs, K., Stock, N., Ross, C., Andrejeva, J., Hilton, L., Skinner, M., Randall, R., Goodbourn, S., 2007. MDA-5, but not RIG-I, is a common target for paramyxovirus V proteins. Virology 359, 190–200.

Colonna, M., Krug, A., Cella, M., 2002. Interferon-producing cells: on the front line in immune responses against pathogens. Curr. Opin. Immunol. 14, 373–379.

Dalod, M., Salazar-Mather, T.P., Malmgaard, L., Lewis, C., Asselin-Paturel, C., Briere, F., Trinchieri, G., Biron, C.A., 2002. Interferon alpha/beta and interleukin 12 responses to viral infections: pathways regulating dendritic cell cytokine expression in vivo. J. Exp. Med. 195, 517–528.

Dalod, M., Hamilton, T., Salomon, R., Salazar-Mather, T.P., Henry, S.C., Hamilton, J.D., Biron, C.A., 2003. Dendritic cell responses to early murine cytomegalovirus infection: subset functional specialization and differential regulation by interferon alpha/beta. J. Exp. Med. 197, 885–898.

Delale, T., Paquin, A., Asselin-Paturel, C., Dalod, M., Brizard, G., Bates, E.E., Kastner, P., Chan, S., Akira, S., Vicari, A., Biron, C.A., Trinchieri, G., Brière, F., 2005. MyD88-dependent and -independent murine cytomegalovirus sensing for IFN-alpha release and initiation of immune responses in vivo. J. Immunol. 175, 6723–6732.

Diebold, S.S., Montoya, M., Unger, H., Alexopoulou, L., Roy, P., Haswell, L.E., Al-Shamkhani, A., Flavell, R., Borrow, P., Reis e Sousa, C., 2003. Viral infection switches non-plasmacytoid dendritic cells into high interferon producers. Nature 424, 324–328.

Diebold, S.S., Kaisho, T., Hemmi, H., Akira, S., Reis e Sousa, C., 2004. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 303, 1529–1531.

Edelmann, K.H., Richardson-Burns, S., Alexopoulou, L., Tyler, K.L., Flavell, R.A., Oldstone, M.B., 2004. Does Toll-like receptor 3 play a biological role in virus infections? Virology 322, 231–238.

Edwards, A.D., Diebold, S.S., Slack, E.M., Tomizawa, H., Hemmi, H., Kaisho, T., Akira, S., Reis e Sousa, C., 2003. Toll-like receptor expression in murine DC subsets: lack of TLR7 expression by CD8 alpha+ DC correlates with unresponsiveness to imidazoquinolines. Eur. J. Immunol. 33, 827–833.

Fitzgerald, K.A., McWhirter, S.M., Faia, K.L., Rowe, D.C., Latz, E., Golenbock, D.T., Coyle, A.J., Liao, S.M., Maniatis, T., 2003. IKKepsilon and TBK1 are essential

components of the IRF3 signaling pathway. Nat. Immunol. 4, 491–496.

Garcia-Sastre, A., 2004. Identification and characterization of viral antagonists of type I interferon in negative-strand RNA viruses. Curr. Top. Microbiol. Immunol. 283, 249–280.

Garcia-Sastre, A., Egorov, A., Matassov, D., Brandt, S., Levy, D.E., Durbin, J.E., Palese, P., Muster, T., 1998. Influenza A virus lacking the NS1 gene replicates in interferon-deficient systems. Virology 252, 324–330.

Gitlin, L., Barchet, W., Gilfillan, S., Cella, M., Beutler, B., Flavell, R.A., Diamond, M.S., Colonna, M., 2006. Essential role of mda-5 in type I IFN responses to polyriboinosinic:polyribocytidylic acid and encephalomyocarditis picornavirus. Proc. Natl. Acad. Sci. USA 103, 8459–8464.

Gorden, K.B., Gorski, K.S., Gibson, S.J., Kedl, R.M., Kieper, W.C., Qiu, X., Tomai, M.A., Alkan, S.S., Vasilakos, J.P., 2005. Synthetic TLR agonists reveal functional differences between human TLR7 and TLR8. J. Immunol. 174, 1259–1268.

Guiducci, C., Ott, G., Chan, J.H., Damon, E., Calacsan, C., Matray, T., Lee, K.D., Coffman, R.L., Barrat, F.J., 2006. Properties regulating the nature of the plasmacytoid dendritic cell response to Toll-like receptor 9 activation. J. Exp. Med. 203, 1999–2008.

Guo, B., Cheng, G., 2007. Modulation of the interferon antiviral response by the TBK1/IKKI adaptor protein tank. J. Biol. Chem. 282, 11817–11826.

Hartmann, G., Battiany, J., Poeck, H., Wagner, M., Kerkmann, M., Lubenow, N., Rothenfusser, S., Endres, S., 2003. Rational design of new CpG oligonucleotides that combine B cell activation with high IFN-alpha induction in plasmacytoid dendritic cells. Eur. J. Immunol. 33, 1633–1641.

Heil, F., Hemmi, H., Hochrein, H., Ampenberger, F., Kirschning, C., Akira, S., Lipford, G., Wagner, H., Bauer, S., 2004. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 303, 1526–1529.

Hemmi, H., Kaisho, T., Takeuchi, O., Sato, S., Sanjo, H., Hoshino, K., Horiuchi, T., Tomizawa, H., Takeda, K., Akira, S., 2002. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. Nat. Immunol. 3, 196–200.

Hochrein, H., Schlatter, B., O'Keeffe, M., Wagner, C., Schmitz, F., Schiemann, M., Bauer, S., Suter, M., Wagner, H., 2004. Herpes simplex virus type-1 induces IFN-alpha production via Toll-like receptor 9-dependent and -independent pathways. Proc. Natl. Acad. Sci. USA 101, 11416–11421.

Hoebe, K., Du, X., Georgel, P., Janssen, E., Tabeta, K., Kim, S.O., Goode, J., Lin, P., Mann, N., Mudd, S., Crozat, K., Sovath, S., Han, J., Beutler, B., 2003. Identification of Lps2 as a key transducer of MyD88-independent TIR signalling. Nature 424, 743–748.

Honda, K., Taniguchi, T., 2006. IRFs: master regulators of signalling by Toll-like receptors and cytosolic pattern-recognition receptors. Nat. Rev. Immunol. 6, 644–658.

Honda, K., Yanai, H., Mizutani, T., Negishi, H., Shimada, N., Suzuki, N., Ohba, Y., Takaoka, A., Yeh, W.C., Taniguchi, T., 2004. Role of a transductional-transcriptional processor complex involving MyD88 and IRF-7 in Toll-like receptor signaling. Proc. Natl. Acad. Sci. USA 101, 15416–15421.

Honda, K., Ohba, Y., Yanai, H., Negishi, H., Mizutani, T., Takaoka, A., Taya, C., Taniguchi, T., 2005a. Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. Nature 434, 1035–1040.

Honda, K., Yanai, H., Negishi, H., Asagiri, M., Sato, M., Mizutani, T., Shimada, N., Ohba, Y., Takaoka, A., Yoshida, N., Taniguchi, T., 2005b. IRF-7 is the master regulator of type-I interferon-dependent immune responses. Nature 434, 772–777.

Hornung, V., Guenthner-Biller, M., Bourquin, C., Ablasser, A., Schlee, M., Uematsu, S., Noronha, A., Manoharan, M., Akira, S., de Fougerolles, A., Endres, S., Hartmann, G., 2005. Sequence-specific potent induction of IFN-alpha by short interfering RNA in plasmacytoid dendritic cells through TLR7. Nat. Med. 11, 263–270.

Hornung, V., Ellegast, J., Kim, S., Brzozka, K., Jung, A., Kato, H., Poeck, H., Akira, S., Conzelmann, K.K., Schlee, M., Endres, S., Hartmann, G., 2006. 5′-Triphosphate RNA is the ligand for RIG-I. Science 314, 994–997.

Hoshino, K., Sugiyama, T., Matsumoto, M., Tanaka, T., Saito, M., Hemmi, H., Ohara, O., Akira, S., Kaisho, T., 2006. IkappaB kinase-alpha is critical for interferon-alpha production induced by Toll-like receptors 7 and 9. Nature 440, 949–953.

Inohara, N., Nunez, G., 2003. NODs: intracellular proteins involved in inflammation and apoptosis. Nat. Rev. Immunol. 3, 371–382.

Ishii, K.J., Coban, C., Kato, H., Takahashi, K., Torii, Y., Takeshita, F., Ludwig, H., Sutter, G., Suzuki, K., Hemmi, H., Sato, S., Yamamoto, M., Uematsu, S., Kawai, T., Takeuchi, O., Akira, S., 2006. A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA. Nat. Immunol. 7, 40–48.

Izaguirre, A., Barnes, B.J., Amrute, S., Yeow, W.S., Megjugorac, N., Dai, J., Feng, D., Chung, E., Pitha, P.M., Fitzgerald-Bocarsly, P., 2003. Comparative analysis of IRF and IFN-alpha expression in human plasmacytoid and monocyte-derived dendritic cells. J. Leukoc. Biol. 74, 1125–1138.

Johnson, C.L., Owen, D.M., Gale Jr., M., 2007. Functional and therapeutic analysis of hepatitis C virus NS3/4A protease control of antiviral immune defense. J. Biol. Chem. 282, 10792–10803.

Jurk, M., Heil, F., Vollmer, J., Schetter, C., Krieg, A.M., Wagner, H., Lipford, G., Bauer, S., 2002. Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. Nat. Immunol. 3, 499.

Kang, D.C., Gopalkrishnan, R.V., Wu, Q., Jankowsky, E., Pyle, A.M., Fisher, P.B., 2002. MDA-5: An interferon-inducible putative RNA helicase with double-stranded RNA-dependent ATPase activity and melanoma growth-suppressive properties. Proc. Natl. Acad. Sci. USA 99, 637–642.

Kato, H., Sato, S., Yoneyama, M., Yamamoto, M., Uematsu, S., Matsui, K., Tsujimura, T., Takeda, K., Fujita, T., Takeuchi, O., Akira, S., 2005. Cell type-specific involvement of RIG-I in antiviral response. Immunity 23, 19–28.

Kato, H., Takeuchi, O., Sato, S., Yoneyama, M., Yamamoto, M., Matsui, K., Uematsu, S., Jung, A., Kawai, T., Ishii, K.J., Yamaguchi, O., Otsu, K., Tsujimura, T., Koh, C.S., Reis e Sousa, C., Matsuura, Y., Fujita, T., Akira, S., 2006. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 441, 101–105.

Kawai, T., Sato, S., Ishii, K.J., Coban, C., Hemmi, H., Yamamoto, M., Terai, K., Matsuda, M., Inoue, J., Uematsu, S., Takeuchi, O., Akira, S., 2004. Interferon-alpha induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. Nat. Immunol. 5, 1061–1068.

Kawai, T., Takahashi, K., Sato, S., Coban, C., Kumar, H., Kato, H., Ishii, K.J., Takeuchi, O., Akira, S., 2005. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. Nat. Immunol. 6, 981–988.

Kerkmann, M., Rothenfusser, S., Hornung, V., Towarowski, A., Wagner, M., Sarris, A., Giese, T., Endres, S., Hartmann, G., 2003. Activation with CpG-A and CpG-B oligonucleotides reveals two distinct regulatory pathways of type I IFN synthesis in human plasmacytoid dendritic cells. J. Immunol. 170, 4465–4474.

Kerkmann, M., Costa, L.T., Richter, C., Rothenfusser, S., Battiany, J., Hornung, V., Johnson, J., Englert, S., Ketterer, T., Heckl, W., Thalhammer, S., Endres, S., Hartmann, G., 2005. Spontaneous formation of nucleic acid-based nanoparticles is responsible for high interferon-alpha induction by CpG-A in plasmacytoid dendritic cells. J. Biol. Chem. 280, 8086–8093.

Kovacsovics, M., Martinon, F., Micheau, O., Bodmer, J.L., Hofmann, K., Tschopp, J., 2002. Overexpression of Helicard, a CARD-containing helicase cleaved during apoptosis, accelerates DNA degradation. Curr. Biol. 12, 838–843.

Krug, A., Rothenfusser, S., Hornung, V., Jahrsdorfer, B., Blackwell, S., Ballas, Z.K., Endres, S., Krieg, A.M., Hartmann, G., 2001. Identification of CpG oligonucleotide sequences with high induction of IFN-alpha/beta in plasmacytoid dendritic cells. Eur. J. Immunol. 31, 2154–2163.

Krug, A., French, A.R., Barchet, W., Fischer, J.A., Dzionek, A., Pingel, J.T., Orihuela, M.M., Akira, S., Yokoyama, W.M., Colonna, M., 2004a. TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. Immunity 21, 107–119.

Krug, A., Luker, G.D., Barchet, W., Leib, D.A., Akira, S., Colonna, M., 2004b. Herpes simplex virus type 1 activates murine natural interferon-producing cells through toll-like receptor 9. Blood 103, 1433–1437.

Kumar, H., Kawai, T., Kato, H., Sato, S., Takahashi, K., Coban, C., Yamamoto, M., Uematsu, S., Ishii, K.J., Takeuchi, O., Akira, S., 2006. Essential role of IPS-1 in innate immune responses against RNA viruses. J. Exp. Med. 203, 1795–1803.

Le Goffic, R., Balloy, V., Lagranderie, M., Alexopoulou, L., Escriou, N., Flavell, R., Chignard, M., Si-Tahar, M., 2006. Detrimental contribution of the Toll-like receptor (TLR)3 to influenza A virus-induced acute pneumonia. PLoS Pathog. 2, e53.

Le Goffic, R., Pothlichet, J., Vitour, D., Fujita, T., Meurs, E., Chignard, M., Si-Tahar, M., 2007. Cutting edge: Influenza A virus activates TLR3-dependent inflammatory and RIG-I-dependent antiviral responses in human lung epithelial cells. J. Immunol. 178, 3368–3372.

Lee, H.K., Lund, J.M., Ramanathan, B., Mizushima, N., Iwasaki, A., 2007. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. Science 315, 1398–1401.

Li, X.D., Sun, L., Seth, R.B., Pineda, G., Chen, Z.J., 2005. Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. Proc. Natl. Acad. Sci. USA 102, 17717–17722.

Loo, Y.M., Owen, D.M., Li, K., Erickson, A.K., Johnson, C.L., Fish, P.M., Carney, D.S., Wang, T., Ishida, H., Yoneyama, M., Fujita, T., Saito, T., Lee, W.M., Hagedorn, C.H., Lau, D.T., Weinman, S.A., Lemon, S.M., Gale Jr., M., 2006. Viral and therapeutic control of IFN-beta promoter stimulator 1 during hepatitis C virus infection. Proc. Natl. Acad. Sci. USA 103, 6001–6006.

Lopez, C.B., Moltedo, B., Alexopoulou, L., Bonifaz, L., Flavell, R.A., Moran, T.M., 2004. TLR-independent induction of dendritic cell maturation and adaptive immunity by negative-strand RNA viruses. J. Immunol. 173, 6882–6889.

Lund, J., Sato, A., Akira, S., Medzhitov, R., Iwasaki, A., 2003. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. J. Exp. Med. 198, 513–520.

Lund, J.M., Alexopoulou, L., Sato, A., Karow, M., Adams, N.C., Gale, N.W., Iwasaki, A., Flavell, R.A., 2004. Recognition of single-stranded RNA viruses by Toll-like receptor 7. Proc. Natl. Acad. Sci. USA 101, 5598–5603.

Lund, J.M., Linehan, M.M., Iijima, N., Iwasaki, A., 2006. Cutting edge: plasmacytoid dendritic cells provide innate immune protection against mucosal viral infection in situ. J. Immunol. 177, 7510–7514.

Matsuda, A., Suzuki, Y., Honda, G., Muramatsu, S., Matsuzaki, O., Nagano, Y., Doi, T., Shimotohno, K., Harada, T., Nishida, E., Hayashi, H., Sugano, S., 2003. Large-scale identification and characterization of human genes that activate NF-kappaB and MAPK signaling pathways. Oncogene 22, 3307–3318.

Matsumoto, M., Funami, K., Tanabe, M., Oshiumi, H., Shingai, M., Seto, Y., Yamamoto, A., Seya, T., 2003. Subcellular localization of Toll-like receptor 3 in human dendritic cells. J. Immunol. 171, 3154–3162.

Means, T.K., Latz, E., Hayashi, F., Murali, M.R., Golenbock, D.T., Luster, A.D., 2005. Human lupus autoantibody-DNA complexes activate DCs through cooperation of CD32 and TLR9. J. Clin. Invest. 115, 407–417.

Meylan, E., Burns, K., Hofmann, K., Blancheteau, V., Martinon, F., Kelliher, M., Tschopp, J., 2004. RIP1 is an essential mediator of Toll-like receptor 3-induced NF-kappa B activation. Nat. Immunol. 5, 503–507.

Meylan, E., Curran, J., Hofmann, K., Moradpour, D., Binder, M., Bartenschlager, R., Tschopp, J., 2005. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature 437, 1167–1172.

Meylan, E., Tschopp, J., Karin, M., 2006. Intracellular pattern recognition receptors in the host response. Nature 442, 39–44.

Mibayashi, M., Martinez-Sobrido, L., Loo, Y.M., Cardenas, W.B., Gale Jr., M., Garcia-Sastre, A., 2007. Inhibition of retinoic acid-inducible gene I-mediated induction of beta interferon by the NS1 protein of influenza A virus. J. Virol. 81, 514–524.

Moseman, E.A., Liang, X., Dawson, A.J., Panoskaltsis-Mortari, A., Krieg, A.M., Liu, Y.J., Blazar, B.R., Chen, W., 2004. Human plasmacytoid dendritic cells activated by CpG oligodeoxynucleotides induce the generation of CD4+CD25+ regulatory T cells. J. Immunol. 173, 4433–4442.

Negishi, H., Fujita, Y., Yanai, H., Sakaguchi, S., Ouyang, X., Shinohara, M., Takayanagi, H., Ohba, Y., Taniguchi, T., Honda, K., 2006. Evidence for licensing of IFN-gamma-induced IFN regulatory factor 1 transcription factor by MyD88 in Toll-like receptor-dependent gene induction program. Proc. Natl. Acad. Sci. USA 103, 15136–15141.

Orange, J.S., Biron, C.A., 1996a. An absolute and restricted requirement for IL-12 in natural killer cell IFN-gamma production and antiviral defense. Studies of natural killer and T cell responses in contrasting viral infections. J. Immunol. 156, 1138–1142.

Orange, J.S., Biron, C.A., 1996b. Characterization of early IL-12, IFN-alphabeta, and TNF effects on antiviral state and NK cell responses during murine cytomegalovirus infection. J. Immunol. 156, 4746–4756.

Perussia, B., Fanning, V., Trinchieri, G., 1985. A leukocyte subset bearing HLA-DR antigens is responsible for in vitro alpha interferon production in response to viruses. Nat. Immun. Cell. Growth Regul. 4, 120–137.

Picard, C., Puel, A., Bonnet, M., Ku, C.L., Bustamante, J., Yang, K., Soudais, C., Dupuis, S., Feinberg, J., Fieschi, C., Elbim, C., Hitchcock, R., Lammas, D., Davies, G., Al-Ghonaium, A., Al-Rayes, H., Al-Jumaah, S., Al-Hajjar, S., Al-Mohsen, I.Z., Frayha, H.H., Rucker, R., Hawn, T.R., Aderem, A., Tufenkeji, H., Haraguchi, S., Day, N.K., Good, R.A., Gougerot-Pocidalo, M.A., Ozinsky, A., Casanova, J.L., 2003. Pyogenic bacterial infections in humans with IRAK-4 deficiency. Science 299, 2076–2079.

Pichlmair, A., Schulz, O., Tan, C.P., Naslund, T.I., Liljestrom, P., Weber, F., Reis e Sousa, C., 2006. RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates. Science 314, 997–1001.

Ronnblom, L., Ramstedt, U., Alm, G.V., 1983. Properties of human natural interferon-producing cells stimulated by tumor cell lines. Eur. J. Immunol. 13, 471–476.

Rothenfusser, S., Goutagny, N., DiPerna, G., Gong, M., Monks, B.G., Schoenemeyer, A., Yamamoto, M., Akira, S., Fitzgerald, K.A., 2005. The RNA helicase Lgp2 inhibits TLR-independent sensing of viral replication by retinoic acid-inducible gene-I. J. Immunol. 175, 5260–5268.

Rudd, B.D., Smit, J.J., Flavell, R.A., Alexopoulou, L., Schaller, M.A., Gruber, A., Berlin, A.A., Lukacs, N.W., 2006. Deletion of TLR3 alters the pulmonary immune environment and mucus production during respiratory syncytial virus infection. J. Immunol. 176, 1937–1942.

Saha, S.K., Pietras, E.M., He, J.Q., Kang, J.R., Liu, S.Y., Oganesyan, G., Shahangian, A., Zarnegar, B., Shiba, T.L., Wang, Y., Cheng, G., 2006. Regulation of antiviral responses by a direct and specific interaction between TRAF3 and Cardif. EMBO J. 25, 3257–3263.

Saito, T., Hirai, R., Loo, Y.M., Owen, D., Johnson, C.L., Sinha, S.C., Akira, S., Fujita, T., Gale Jr., M., 2007. Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. Proc. Natl. Acad. Sci. USA 104, 582–587.

Salio, M., Palmowski, M.J., Atzberger, A., Hermans, I.F., Cerundolo, V., 2004. CpG-matured murine plasmacytoid dendritic cells are capable of in vivo priming of functional CD8 T cell responses to endogenous but not exogenous antigens. J. Exp. Med. 199, 567–579.

Sato, S., Sugiyama, M., Yamamoto, M., Watanabe, Y., Kawai, T., Takeda, K., Akira, S., 2007. Toll/IL-1 receptor domain-containing adaptor inducing IFN-beta (TRIF) associates with TNF receptor-associated factor 6 and TANK-binding kinase 1, and activates two distinct transcription factors, NF-kappa B and IFN-regulatory factor-3, in the Toll-like receptor signaling. J. Immunol. 171, 4304–4310.

Schlecht, G., Garcia, S., Escriou, N., Freitas, A.A., Leclerc, C., Dadaglio, G., 2004. Murine plasmacytoid dendritic cells induce effector/memory CD8+ T-cell responses in vivo after viral stimulation. Blood 104, 1808–1815.

Schmitz, F., Heit, A., Guggemoos, S., Krug, A., Mages, J., Schiemann, M., Adler, H., Drexler, I., Haas, T., Lang, R., Wagner, H., 2007. Interferon-regulatory-factor 1 controls Toll-like receptor 9-mediated IFN-beta production in myeloid dendritic cells. Eur. J. Immunol. 37, 315–327.

Schulz, O., Diebold, S.S., Chen, M., Naslund, T.I., Nolte, M.A., Alexopoulou, L., Azuma, Y.T., Flavell, R.A., Liljestrom, P., Reis e Sousa, C., 2005. Toll-like receptor 3 promotes cross-priming to virus-infected cells. Nature 433, 887–892.

Seth, R.B., Sun, L., Ea, C.K., Chen, Z.J., 2005. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. Cell 122, 669–682.

Sharma, S., tenOever, B.R., Grandvaux, N., Zhou, G.P., Lin, R., Hiscott, J., 2003. Triggering the interferon antiviral response through an IKK-related pathway. Science 300, 1148–1151.

Shinohara, M.L., Lu, L., Bu, J., Werneck, M.B., Kobayashi, K.S., Glimcher, L.H., Cantor, H., 2006. Osteopontin expression is essential for interferon-alpha production by plasmacytoid dendritic cells. Nat. Immunol. 7, 498–506.

Siegal, F.P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P.A., Shah, K., Ho, S., Antonenko, S., Liu, Y.J., 1999. The nature of the principal type 1 interferon-producing cells in human blood. Science 284, 1835–1837.

Stetson, D.B., Medzhitov, R., 2006. Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. Immunity 24, 93–103.

Sumpter Jr., R., Loo, Y.M., Foy, E., Li, K., Yoneyama, M., Fujita, T., Lemon, S.M., Gale Jr., M., 2005. Regulating intracellular antiviral defense and permissiveness to

hepatitis C virus RNA replication through a cellular RNA helicase, RIG-I. J. Virol. 79, 2689–2699.

Sun, Q., Sun, L., Liu, H.H., Chen, X., Seth, R.B., Forman, J., Chen, Z.J., 2006. The specific and essential role of MAVS in antiviral innate immune responses. Immunity 24, 633–642.

Tabeta, K., Georgel, P., Janssen, E., Du, X., Hoebe, K., Crozat, K., Mudd, S., Shamel, L., Sovath, S., Goode, J., Alexopoulou, L., Flavell, R.A., Beutler, B., 2004. Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. Proc. Natl. Acad. Sci. USA 101, 3516–3521.

Tabeta, K., Hoebe, K., Janssen, E.M., Du, X., Georgel, P., Crozat, K., Mudd, S., Mann, N., Sovath, S., Goode, J., Shamel, L., Herskovits, A.A., Portnoy, D.A., Cooke, M., Tarantino, L.M., Wiltshire, T., Steinberg, B.E., Grinstein, S., Beutler, B., 2006. The Unc93b1 mutation 3d disrupts exogenous antigen presentation and signaling via Toll-like receptors 3, 7 and 9. Nat. Immunol. 7, 156–164.

Takaoka, A., Wang, Z., Choi, M.K., Yanai, H., Negishi, H., Ban, T., Lu, Y., Miyagishi, M., Kodama, T., Honda, K., Ohba, Y., Taniguchi, T., 2007. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. Nature 448, 501–505.

Talon, J., Horvath, C.M., Polley, R., Basler, C.F., Muster, T., Palese, P., Garcia-Sastre, A., 2000. Activation of interferon regulatory factor 3 is inhibited by the influenza A virus NS1 protein. J. Virol. 74, 7989–7996.

Uematsu, S., Sato, S., Yamamoto, M., Hirotani, T., Kato, H., Takeshita, F., Matsuda, M., Coban, C., Ishii, K.J., Kawai, T., Takeuchi, O., Akira, S., 2005. Interleukin-1 receptor-associated kinase-1 plays an essential role for Toll-like receptor (TLR)7- and TLR9-mediated interferon-{alpha} induction. J. Exp. Med. 201, 915–923.

Verthelyi, D., Ishii, K.J., Gursel, M., Takeshita, F., Klinman, D.M., 2001. Human peripheral blood cells differentially recognize and respond to two distinct CPG motifs. J. Immunol. 166, 2372–2377.

Wang, T., Town, T., Alexopoulou, L., Anderson, J.F., Fikrig, E., Flavell, R.A., 2004. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. Nat. Med. 10, 1366–1373.

Xu, L.G., Wang, Y.Y., Han, K.J., Li, L.Y., Zhai, Z., Shu, H.B., 2005. VISA is an adapter protein required for virus-triggered IFN-beta signaling. Mol. Cell 19, 727–740.

Yamamoto, M., Sato, S., Hemmi, H., Hoshino, K., Kaisho, T., Sanjo, H., Takeuchi, O., Sugiyama, M., Okabe, M., Takeda, K., Akira, S., 2003. Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science 301, 640–643.

Yoneyama, M., Kikuchi, M., Natsukawa, T., Shinobu, N., Imaizumi, T., Miyagishi, M., Taira, K., Akira, S., Fujita, T., 2004. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat. Immunol. 5, 730–737.

Yoneyama, M., Kikuchi, M., Matsumoto, K., Imaizumi, T., Miyagishi, M., Taira, K., Foy, E., Loo, Y.M., Gale Jr., M., Akira, S., Yonehara, S., Kato, A., Fujita, T., 2005. Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. J. Immunol. 175, 2851–2858.

Zhang, X., Wang, C., Schook, L.B., Hawken, R.J., Rutherford, M.S., 2000. An RNA helicase, RHIV-1, induced by porcine reproductive and respiratory syndrome virus (PRRSV) is mapped on porcine chromosome 10q13. Microb. Pathog. 28, 267–278.