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TLR-Independent Induction of Dendritic Cell Maturation and Adaptive Immunity by Negative-Strand RNA Viruses

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TLR signaling leads to dendritic cell (DC) maturation and immunity to diverse pathogens. The stimulation of TLRs by conserved viral structures is the only described mechanism leading to DC maturation after a virus infection. In this report, we demonstrate that mouse myeloid DCs mature normally after in vivo and in vitro infection with Sendai virus (SeV) in the absence of TLR3, 7, 8, or 9 signaling. DC maturation by SeV requires virus replication not necessary for TLR-mediated triggering. Moreover, DCs deficient in TLR signaling efficiently prime for Th1 immunity after infection with influenza or SeV, generating IFN-γ-producing T cells, CTLs and antiviral Abs. We have previously demonstrated that SeV induces DC maturation independently of the presence of type I IFN, which has been reported to mature DCs in a TLR-independent manner. The data presented here provide evidence for the existence of a novel intracellular pathway independent of TLR-mediated signaling responsible for live virus triggering of DC maturation and demonstrate its critical role in the onset of antiviral immunity. The revelation of this pathway should stimulate invigorating research into the mechanism for virus-induced DC maturation and immunity. The Journal of Immunology, 2004, 173: 6882–6889.

Dendritic cells (DCs) mediate the transition from innate to adaptive immunity by undergoing a major shift in their maturation state that involves the up-regulation of costimulatory molecules, and the secretion of proinflammatory cytokines necessary for the differentiation of effector and memory T cells and B cells (1). The binding of pathogen-associated molecular patterns (PAMPs) to an assortment of receptors expressed on the DCs, known as TLRs, constitute the primary mechanism described to trigger DC maturation (2). To date, more than 10 different TLRs have been identified in vertebrates, with specificities for all major families of microbes (3).

A role for TLRs in monitoring viral infections has been established based on the discovery of TLRs responsive to viral elements. Double-stranded RNA (dsRNA), a viral PAMP generated during virus replication, binds and activates TLR3 and CpG motifs found in the DNA of HSV-activator TLR9 (5, 6). Moreover, single-stranded viral RNA (ssRNA), representing the genomic material of many different viruses, triggers the activation of TLR7 and TLR8 (7–9). Viral envelope proteins (e.g., the respiratory syncytial virus (RSV) fusion protein) have also been shown to trigger TLR activation (10–13). However, the lack of a common structure or PAMP within these proteins questions the role of these interactions in the generic recognition of viruses.

Despite the triggering of DC maturation through the binding of viral components to TLRs, the role of TLR signaling in eliciting antiviral adaptive immune responses is unknown. In humans, the virus-responsive TLR7, TLR8, and TLR9 are expressed in a restricted subset of DCs, the plasmacytoid DCs (pDCs), which are specialized in secreting large amounts of type I IFN after virus recognition (14, 15). Controversy exists regarding the ability of these cells to stimulate naive T cells. Although CpG-matured pDCs are able to present Ag and stimulate naive T cells in vitro as well as in vivo (16–18), evidence also exists suggesting that pDCs poorly present Ag and are unable to prime naive T cells (16, 19). In addition, all virus-related TLRs can be stimulated by inactivated viruses or viral RNA surrogates (4, 7, 8), but live virus infection is needed for the optimal induction of DC maturation and immunity against ssRNA viruses (20, 21). These observations argue that the predominant role for TLR signaling during virus infection might be to elicit innate immune responses rather than to generate adaptive immunity.

Thus, we set out to investigate whether the induction of DC maturation and adaptive immunity to viral infections could be triggered independently of TLR signaling. To do this, we took advantage of the paramyxovirus Sendai virus (SeV) strain Cantell, a particularly strong inducer of mouse DC maturation (22). We have previously demonstrated that the potent DC maturation-inducing activity of SeV is not dependent on the secretion of the antiviral cytokine type I IFN, which has been related to the amplification and preservation of DC maturation after infection with other viruses, such as Newcastle disease virus (21). SeV allowed us to visualize the requirements for DC maturation after infection with live viruses independently of the contribution of type I IFN signaling. Our data demonstrate that TLR3 or the TLR adaptor protein MyD88, necessary for the signaling of most TLRs, are not
essential for the induction of costimulatory molecule expression or the secretion of cytokines by mature DCs after in vitro or in vivo infection and that their absence has no significant impact on the generation of antiviral Th1 immunity in mice.

Materials and Methods

Viruses and mice

SeV Cantell and influenza A virus were grown, purified, and inactivated as described (20, 22). RSV (A2 strain) was grown in mycoplasma-free Vero cells. C57BL/6 and MHC class I−/− mice were purchased from Taconic Farms (Germantown, NY). MyD88−/− mice were kindly provided by Dr. S. Akira (Osaka University, Osaka, Japan) and Dr. R. Steinman (Rockefeller University, New York, NY). Age- and sex-matched animals were used in all experiments. The animals were housed in pathogen-free conditions and the experiments were performed according to institutionally approved protocols.

Bone marrow-derived DC (BMDC) preparation and treatment

BMDCs were prepared as previously described (22) and infected with either influenza (multiplicity of infection, (MOI) of 10), SeV (MOI of 0.6–10) or RSV (MOI of 2). Control BMDCs were mock infected or incubated overnight with 10 ng/ml LPS (Sigma-Aldrich, St. Louis, MO) or 50 μg/ml poly(I:C) (Amer sham Biosciences, Bucks, U.K.). For maturation studies the cells were incubated overnight at 1 × 10^6 cells/ml in media containing 1% normal mouse serum. Removal of remaining extracellular virus particles was performed as described using neutralizing anti-PR8 influenza (PY102 and PY210) or anti-SeV Abs (obtained from Dr. A. Portner, St. Jude Children’s Hospital, Memphis, TN (23)).

DC isolation from lymph nodes and staining

Lung-draining lymph nodes were harvested 48 h after mice infection. Tissues were incubated 20 min with collagenase (Liberase Blendzymes; Roche, Indianapolis, IN). Washed single cell suspensions were incubated for 4 h with brefeldin A (BD Biosciences, San Diego, CA). Cells were stained with allophycocyanin-conjugated anti-CD11c and FITC-labeled anti-CD86 Abs (BD Biosciences). Intracellular staining for IL-12 was performed using the Cytofix/Cytoperm kit from BD Biosciences and PE-conjugated anti-IL-12 Ab (BD Biosciences). Infected, mock-infected, or LPS-treated BMDCs were stained 24 h after treatment with FITC-conjugated anti-CD86 or anti-CD80 Abs (BD Biosciences). Flow cytometry was performed in a XLS-Coulter station (Beckman Coulter, Miami, FL). Data was analyzed with FlowJo software.

Mice infection, immunization, and Ab detection

Mice were exposed for 30 min to ~900 influenza virus particles/min/cm^3 of air in an infection chamber (Glas-Col, Terre Haute, IN). For immunizations, mice were injected i.p. with 4 × 10^5 DCs or with 10^7 TCID_50 of influenza or SeV. Detection of antiviral IgG1 and IgG2b Abs in mouse serum was performed by capture ELISA as described (20).

Spleen cell culture and cytotoxicity

Stimulator splenocytes from naive C57BL/6 or MyD88−/− mice were infected with influenza or SeV at an MOI of 40 for 45 min at 37°C and cultured with spleen cells from immunized animals at a 1:10 stimulator to responder ratio for SeV-infected cells and a 1:1 stimulator to responder ratio for influenza-virus-infected cells (20). Effector cells from secondary in vitro cultures were mixed with 51Cr-labeled infected or mock-infected EL4 target cells as described (20). Supernatants were harvested and gamma radiation was measured. Killing of mock-infected EL4 cells was subtracted from that observed with infected targets.

Cytokine detection

Supernatants from DC and spleen cell cultures were analyzed by Capture ELISA for IL-6, IL-1β, IL-12p40, TNF-α, IL-4 (R&D Systems, Minneapolis, MN), and IFN-γ (PBL Biomedical Laboratories, Piscataway, NJ) following the manufacturer’s protocols. IFN-γ was measured using Ab pairs from BD Biosciences.

In vivo CTL assay and tetramer staining

Splenocytes from naive mice were pulsed at 4 × 10^7 cells/ml with 20 μM SeV nucleoprotein (NP)_{324-332} peptide or without peptide in PBS for 15 min at room temperature. The cells were then labeled at 2 × 10^6 cells/ml with different concentrations of CFSE (Molecular Probes, Eugene, OR; 2.5 or 0.125 μM) at 37°C for 30 min. Labeling was stopped with an equal volume of FBS for 1 min and 1 × 10^7 cells of each peptide pulsed and unpulsed were mixed and injected i.v. into infected and uninfected syngenic mice. After 20 h from the injections, spleens were harvested and single cell suspensions were prepared and analyzed by flow cytometry. Percentage of specific killing was calculated as described (24): 100 − (%

![FIGURE 1. TLR3−/− DCs mature normally after infection with SeV. C57BL/6 (wt) and TLR3−/− DCs (ko) were either infected with SeV or RSV or treated with poly(I:C) or LPS. After 24 h of culture, the cells were stained for CD86 or CD80 expression (filled histogram) and analyzed by flow cytometry (a). Thin line represents isotype controls. Cytokines in the culture supernatants were analyzed after 24 h of culture by capture ELISA (b).](http://www.jimmunol.org/Download/2005/01/1822/FIGURE.jpg)
peptide pulsed in infected mice/uninfected mice)/ (% peptide pulsed in uninfected/uninfected). To determine the percentage of T cells bearing the specific anti-SeV-NP 324–332 peptide H-2Kb receptor, splenocytes or lung single cell suspensions were obtained 10–12 days after infection with SeV and costained with anti-CD8 FITC Ab and PE-labeled H-2Kb tetramers carrying the SeV NP324–332 peptide (National Institutes of Health tetramer core facility).

Statistical analysis
Statistical analysis was performed by using $t$ test, paired two sample analysis.

Results
TLR3 signaling is not required for DC maturation after infection with SeV

The first evidence for viral-induced TLR signaling was the demonstration that the synthetic dsRNA analog poly(I:C), as well as isolated viral dsRNA, could induce the expression of costimulatory molecules on B cells and the secretion of cytokines from APCs through TLR3 signaling (4). Since then, a number of seemingly contradictory studies have attempted to define the role of TLR3 in dsRNA-induced cell activation (8, 21, 25–27). Concurring with reports by others (8, 21, 25), we observe that the up-regulation of costimulatory molecules and the secretion of IFN-$\gamma$ are independent of TLR3 signaling when poly(I:C) is used to treat DCs. In contrast, the secretion of IL-12 and TNF is impaired in the absence of this receptor (Fig. 1).

To test the role of TLR3 in the triggering of DC maturation after live virus infection, DCs were infected with SeV. LPS and RSV were used as controls for TLR3-independent induction of DC maturation as they have been shown to trigger the secretion of cytokines through binding to TLR4 (10, 28). As shown in Fig. 1, normal costimulatory molecule up-regulation and cytokine secretion is observed after SeV infection of TLR3$^{-/-}$ DCs when compared with wild-type DCs, mimicking the effect seen when TLR3-independent stimuli (LPS or RSV) are used. These results demonstrate that TLR3 stimulation is not necessary for the triggering of DC maturation after infection with SeV.

DC maturation after SeV infection is independent of MyD88-mediated signaling

In mice, TLR7, TLR8, and TLR9 are expressed in myeloid DCs, pivotal in the initiation of adaptive immunity (29). To test the requirement for TLR7, 8, or 9 stimulation in the induction of myeloid DC maturation, we took advantage of mice deficient in MyD88, an adaptor protein essential for their signaling (7, 8). MyD88 binds to members of the IL-1R-associated protein kinases and signals further via the TNFR-associated factor-6 (7, 9). MyD88$^{-/-}$ DCs show normal up-regulation of CD80 and CD86 following SeV infection (Fig. 2a). IL-12, IL-6, TNF, and IFN-$\gamma$ secretion by infected DCs is also completely independent of MyD88 after SeV infection (Fig. 2b). Additionally, and in agreement with previous reports (22), the induction of proinflammatory cytokine secretion by SeV-infected DCs depends on virus replication as shown by the absence of cytokines in response to UV-inactivated virus (Fig. 2b). In contrast, the induction of cytokine secretion by the TLR-activators RSV and LPS is completely abolished in MyD88$^{-/-}$ cells and RSV replication is not a requirement for the stimulation of cytokine secretion by DCs (Fig. 2b). The independence of MyD88 signaling for the secretion of type I IFN and other proinflammatory cytokines and for the up-regulation of costimulatory molecules, is observed as early as 6 h postinfection (Fig. 2c and data not shown) and is independent of the dose of SeV used (Fig. 2d and data not shown). These data demonstrate that signaling by the adaptor protein MyD88 is not necessary for the induction of DC maturation after infection with SeV.

FIGURE 2. MyD88$^{-/-}$ DCs mature normally after infection with SeV. C57BL/6 and MyD88$^{-/-}$ DCs were infected with either live or UV-inactivated SeV or RSV, or treatment with LPS. After 24 h of culture, the cells were stained for CD86 or CD80 expression (filled histogram) and analyzed by flow cytometry (a). Thin line represents isotype controls. Cytokines in the culture supernatants were analyzed after 6 (c) or 24 (a, c, and d) h of culture by capture ELISA (b). Data are representative of more than three experiments.
DCs mature normally after SeV infection in MyD88−/− mice

To corroborate our in vitro observations, we analyzed the induction of DC maturation, in vivo, after intranasal infection of MyD88−/− mice with SeV. Two days after infection with influenza or SeV, mature DCs with up-regulated costimulatory molecules and producing proinflammatory cytokines, can be obtained from the lymph nodes of infected animals (Ref. 30 and data not shown). As shown in Fig. 3a, mature DCs with up-regulated CD86 can be seen in the lung-draining lymph nodes of wild-type and MyD88−/− mice 2 days after infection with SeV. Moreover, a similar increase in the expression of IL-12p40/p70 is observed in DCs from wild-type and MyD88−/− mice upon virus infection despite a difference in the basal level of expression of this cytokine among these animals (Fig. 3, b and c). These results demonstrate that following SeV infection, DCs equivalently matured can be distinguished in the lung-draining lymph nodes of wild-type and MyD88−/− mice.

MyD88−/− mice expand IFN-γ secreting cells and CTLs after virus infection

To analyze the role of MyD88 signaling in the development of antiviral immunity, we infected mice with SeV and analyzed the primary generation of CTLs, as well as the development of anti-SeV Abs. Both wild-type and MyD88−/− mice expand CTLs that eliminate target cells carrying the immunodominant SeV peptide (NP324–332), in vivo, 7 days after the infection (Fig. 4a). However, less efficient killing is observed in MyD88−/− mice consistent with a reduction in the number of CD8+ cells bearing SeV-NP-specific TCR, as detected by staining with NP-peptide bearing tetramers (Fig. 4b). Wild-type and MyD88−/− mice develop equivalent amounts of total virus-specific IgG Abs (data not shown) with a slight bias toward the Th2-isotype IgG1 in MyD88−/− mice (Fig. 4c).

Our laboratory has a well-characterized in vivo model for influenza infection (31). We took advantage of this model to study the role of MyD88 signaling in the development of virus-specific humoral and memory cellular responses. After infection with aerosolized live influenza virus, known to induce potent Th1 immunity (31, 32), control and MyD88−/− mice develop equivalent amounts of total virus-specific IgG Abs (data not shown) with a slight bias toward the Th2-isotype IgG1 in MyD88−/− mice (Fig. 4d), similar to that observed in SeV-infected animals (Fig. 4c). Moreover, splenocytes from MyD88−/− and wild-type mice restimulated in vitro with influenza virus 14 days after the infection, secrete comparable levels of the Th1 cytokine IFN-γ together with a remarkable production of the Th2 cytokine IL-4 only by MyD88−/− cells (Fig. 4e). A small reduction in the clearance of both viruses is observed in MyD88−/− animals when compared with wild-type mice 7 days postinfection (data not shown), but both groups of animals show complete recovery from infection as measured by recovery of lost weight and virus clearance from the lungs 10 days postinfection (data not shown). Interestingly, despite the secretion of IL-4 and the Th2-biased Ab production, normal development of memory CTL precursors is observed in MyD88−/− mice (Fig. 4f). Thus, although MyD88−/− mice show minor differences in the in vivo response to both, SeV and influenza virus, a normal immune adaptive response is generated that leads to complete recovery from infection.

Virus-infected DCs deficient in MyD88 signaling induce polarized Th1 immune response in mice

MyD88 is a required element in the signaling through IL-1 and IL-18 receptors, both members of the Toll/IL-1R containing receptor family (33, 34). Therefore, the less efficient development of CD8+ cells bearing SeV-NP-specific TCR (Fig. 4b) and a nonpolarized memory immune response in MyD88−/− mice may result from a failure of these cytokines to promote the differentiation of CD4+ T cells into a Th1 phenotype (35). To unambiguously determine the role of MyD88 in the initiation of antiviral immunity, its function in APCs must be distinguished from its effect in T cells. We immunized mice by adoptively transferring ex vivo infected DCs to distinguish the effect of MyD88 on these cells from that on T cells. DCs, which are abortively infected by influenza and SeV, have been shown to efficiently induce protective antiviral immunity (23). BMDCs were infected with influenza or SeV and injected i.p. into wild-type animals. Both IgG1 and IgG2b Abs are produced when control or MyD88−/−-infected DCs are used for immunization (Fig. 5a). No evidence for a Th2 (IgG1)-biased response is seen when immunizing with infected MyD88−/− DCs as observed in directly immunized MyD88−/− mice (Fig. 4a). In addition, high levels of IFN-γ were secreted by splenocytes from animals immunized with infected wild-type or MyD88−/− DCs (Fig. 5b), corresponding with the development of CTLs (Fig. 5c). We were unable to detect IL-4 production in any of the cultures. Similarly, adoptively transferred TLR3−/− DCs efficiently induced antiviral CTLs when infected with influenza or SeV (data not shown).

To validate this model, we analyzed the contribution of cross-presentation to the immune response generated by this method. This phenomenon occurs when Ag is transferred from dead cells to endogenous cells which present antigenic peptides on their own.
MHC class I molecules and could bias the interpretation of results obtained when DCs are used for immunization (36). Control or MHC class I−/− DCs were infected with SeV and injected into C57BL/6 mice. As shown in Fig. 6, a and b, MHC class I−/− DCs are very inefficient in generating CTL only partially eliminating target cells carrying the immunodominant SeV peptide NP324–332. This correlates with more than a 70% reduction of CD8−/− cells bearing a TCR specific for the SeV NP324–332 peptide as detected by staining with NP324–332-conjugated tetramers (Fig. 6c). These data demonstrate that at least 50% of the CTLs generated after DC immunization are the result of direct presentation of viral Ags by the injected DCs, and allowed us to demonstrate that DCs deficient in TLR3 or MyD88 can induce normal antiviral adaptive immunity in mice.

Discussion
DC maturation, necessary for the transition from innate to adaptive immunity, can be triggered by TLR signaling after recognition of microbial structures. Because a number of TLRs can signal in response to viruses, we sought to determine whether the virus-triggered TLR signaling is essential for the maturation of myeloid DC, and the subsequent induction of antiviral immunity.

TLR7 and TLR8 are receptors for ssRNA, the genetic material of many viruses including those used in this study (7–9). In humans, the expression of these TLRs is restricted among the DC populations to pDCs, and most studies analyzing their role in cell activation by viruses have only focused on this DC subpopulation (7, 8). In mouse, TLR7 and TLR8 are also expressed in myeloid DCs, the primary cells responsible for the induction of adaptive immunity (29). As signaling through these receptors has been shown to be dependent on MyD88, our results using MyD88−/− BMDCs demonstrate that in mice TLR7 and TLR8 are not needed for maturation of myeloid DCs in response to SeV. The failure of inactivated SeV to trigger DC maturation strengthens the argument that TLR7 and TLR8, which have been shown to be stimulated by inactivated virus, are not necessary for myeloid DC maturation. TLR3 that responds to the virus replication intermediary dsRNA, is also expressed on myeloid DCs. This receptor signals by MyD88-dependent and -independent pathways forcing us to investigate its role in the induction of DC maturation by SeV separately from the MyD88-dependent TLRs. Our results demonstrate that TLR3 signaling is not needed for the induction of myeloid DC maturation by SeV and agrees with accumulating data arguing that TLR3 signaling is not necessary for the induction of DC maturation and immunity by other viruses (9, 21, 37). Furthermore, DCs from MyD88−/− and wild-type mice showed identical up-regulation of costimulatory molecules and an increase in IL-12 production following in vivo infection with SeV.
corroborating the dispensable role of MyD88 signaling in the induction of DC maturation after infection with SeV observed in vitro.

To complement these studies, we show that the development of an efficient antiviral adaptive immune response is independent of TLR3 or MyD88-dependent activation. Although a slight Th2 bias in the antiviral immune response is observed in MyD88$^{-/-}$ mice, probably resulting from a deficiency in IL-1 and IL-18 signaling, viral clearance, the production of IFN-$\gamma$ by T cells, and cytotoxic T cell expansion are not significantly different from control animals. The normal functioning of MyD88$^{-/-}$ DCs is supported by experiments adoptively transferring virus-infected MyD88$^{-/-}$ DCs into wild-type mice. No Th2 bias was observed under these conditions and MyD88$^{-/-}$ DCs induced cytokines, Ab and CTL generation are indistinguishable from the animals infected with virus-infected wild-type DCs. Studies using MHC I$^{-/-}$ DCs validated the model system by showing that a significant part of the response observed following adoptive transfer is elicited by the injected cells and not by cross-priming or in vivo uptake of virus or viral proteins. The independence of MyD88 signaling for the normal development of antiviral immunity concurs with the reported normal antiviral immunity in children carrying a mutation in IL-1R-associated protein kinase-4, a crucial downstream element in the signaling pathway that uses the adaptor MyD88 (38).

Altogether, the data presented argue for a TLR-independent pathway for myeloid DC maturation that is dependent on virus replication. The cellular machinery responsible for the induction of myeloid DC maturation after virus infection remains elusive. Although the dsRNA-dependent protein kinase R has been shown to mediate these events upon transfection of poly(IC), we and others have demonstrated that live virus-induced maturation is independent of protein kinase R (21, 22), arguing that the pathway activated by replicating virus may be separate or at least more complex than what is triggered by dsRNA alone. Further studies are required to determine the role of other cellular proteins, such as the TANK (TNFR-associated factor-associated NF-$\kappa$B kinase) binding kinase I, the inhibitor of NF-$\kappa$B kinase $\epsilon$ (39, 40), NOD (nucleotide-binding oligomerization domain) (41), and the recently described RNA helicase retinoic acid inducible gene I (42), which are known to be triggered by dsRNA, in the induction of DC maturation after virus infection.

Additionally, type I IFN produced by all virus-infected cells as a means to contain viral replication and spreading has been shown to be required for optimal DC maturation after infection with Newcastle disease virus (21). We have previously shown that SeV-induced maturation of myeloid DC is independent of exogenous type I IFN (22). In addition, SeV efficiently blocks the IFN signaling pathway in infected cells (43). We have noted that viruses that strongly activate the type I IFN synthesis machinery, such as SeV strain Cantell, are also the best inducers of DC maturation (22) suggesting the existence of shared components in the induction pathways of type I IFN production and DC maturation. The difference in the need for exogenous type I IFN signaling for the complete maturation of DCs after different virus infections may depend on the strength of the viral activation signal. In light of these observations, DCs may require, in some instances, exogenous sources of type I IFN for optimal maturation. Type I IFN released after TLR signaling not reliant on virus replication and therefore not inhibitable by type I IFN antagonists, may fulfill this requirement. This would suggest a novel physiologically relevant role for pDCs as endogenous adjuvants.

Despite our observations, a growing number of examples implicate TLRs in the recognition of diverse viruses, including the binding of the RSV F protein to TLR4 (10) and the measles virus H protein to TLR2 (11). Interestingly, even though these interactions result in activation of APCs and the secretion of cytokines, they do not always result in the development of efficient antiviral immunity. This is well exemplified by the ineffective immune response generated against RSV (44, 45). Moreover, no defined common structure seems to be involved in the binding of these viruses to TLRs leaving open the possibility that these obligatory intracellular pathogens escape or conveniently use the scrutiny of TLRs. Interestingly, RSV induces a MyD88-dependent secretion of cytokines by DCs but fails to up-regulate costimulatory molecules (Fig. 3). This unusual TLR-mediated maturation of DCs by
RSV could be fundamental for the generation of the impaired adaptive immune response against this virus that allows multiple re-infections during childhood.

In conclusion, we have demonstrated that SeV can induce efficient DC maturation and immunity in a TLR3- and MyD88-independent manner. This pathway, which requires live virus, implies a novel mechanism for virus detection and initiation of an antiviral immune response. Its further characterization should allow a better understanding of the mechanisms of viral antagonism that interfere with the generation of immunity, as well as the identification of molecular targets amenable to manipulation for the control of antiviral immune responses and the improvement of vaccination strategies.

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