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Current Opinion in Virology

Innate and adaptive immune responses to viral infection and vaccination

Taiki Aoshi[1,2], Shohei Koyama[3], Kouji Kobiyama[1,2], Shizuo Akira[4] and Ken J Ishii[1,2]

Recent accumulating evidence suggests that the human immune system possesses a variety of innate receptors that recognize, distinguish, and respond to viral infections and to vaccination. These include Toll-like receptors, C-type lectin receptors, RIG-I-like receptors, Nod-like receptors and possibly AIM2-like receptors. However, the precise mechanisms by which these receptors exert their critical roles in the induction of virus-specific adaptive immune responses have not been fully elucidated. In this review, we discuss recent advances in our understanding of the innate immune recognition of viruses and the differential connection to the adaptive immune responses induced by infection or vaccination, with a particular focus on the influenza virus.

Addresses
[1] Laboratory of Adjuvant Innovation, National Institute of Biomedical Innovation, Osaka, Japan
[2] Laboratory of Vaccine Science, WPI Immunology Frontier Research Center, Osaka University, Osaka, Japan
[3] Department of Respiratory Medicine, Tohoku University Graduate School of Medicine, Miyagi, Japan
[4] Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University, Osaka, Japan

Corresponding author: Ishii, Ken J (kenishii@biken.osaka-u.ac.jp)

Current Opinion in Virology 2011, **1**:226–232

This review comes from a themed issue on Vaccines
Edited by Hildegund Ertl and Bali Pulendran

Available online 30th July 2011

1879-6257/$ – see front matter
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DOI 10.1016/j.coviro.2011.07.002

Introduction

Several families of innate immune receptors, including Toll-like receptors (TLRs) [1], C-type lectin receptors [2], RIG-I-like receptors (RLRs) [3], Nod-like receptors (NLRs) [4], and AIM2-like receptors (ALRs) [5] have been identified over the last decade. Generally, these germ-line-encoded receptors recognize 'non-self' molecules derived from a variety of microbes. Some of these receptors also recognize danger signals sent out by damaged cells/tissues [6]. These innate immune receptors are critical for the initiation and regulation of host immune responses against infection and autoimmunity [7]. Furthermore, it is evident that innate immune responses are extremely important for establishing effective adaptive immune responses to infection and vaccination [8•,9,10]; although it is still not clear whether all innate responses contribute equally to the induction of adaptive responses [8•,11•,12•]. In the following sections, we briefly review the current knowledge about virus recognition by innate immune receptors, and discuss the connections between the innate and adaptive immune responses, using influenza virus as an example.

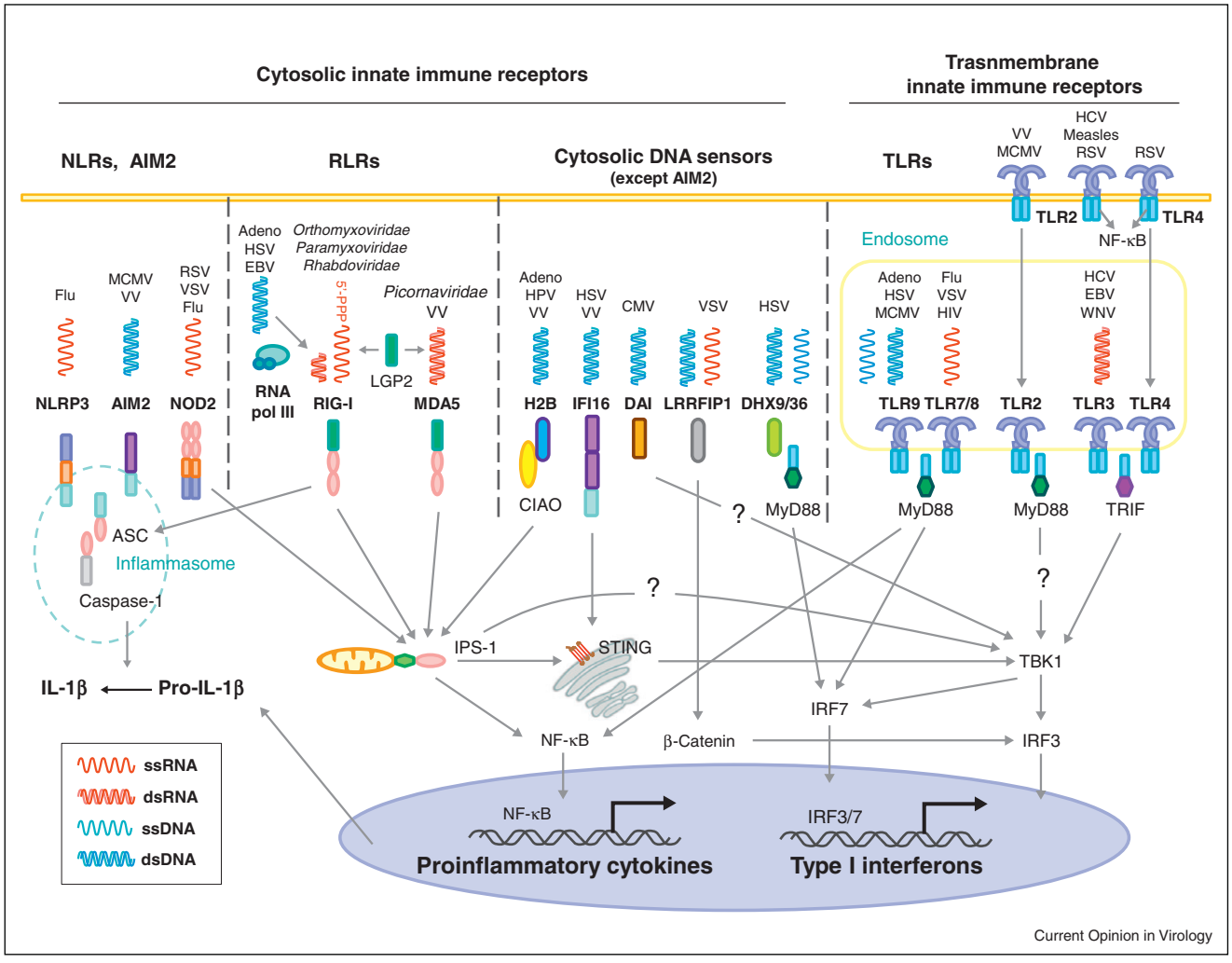
The innate immune system may distinguish between the presence of a virus and viral infection

In addition to bacteria and parasites, viruses are a major cause of infectious diseases. Because of their diverse organ/tissue tropisms, genomic structure (positive or negative stranded, single or double stranded, RNA or DNA) and pathogenic lifecycles, host cells can recognize viruses through a variety of innate immune receptors. Extracellular viruses are detected by transmembrane receptors such as TLRs, and cytosolic viral infections are detected by cytosolic receptors such as RLRs, NLRs, and ALRs (Figure 1). This diverse set of innate receptors may also allow the host immune system to determine viral status — live or dead, replicating or not replicating, pathogenic or non-pathogenic — in a manner similar to that recently proposed for bacterial infection [13]. These innate immune receptors trigger signaling cascades that are generally integrated with innate responses, such as nuclear factor kappa B (NF-κB)-dependent cytokine responses, interferon regulatory factor (IRF)-dependent IFN-α/β responses, and inflammasome/caspase-1-dependent IL-1β responses. IFN-α/β are the major cytokines that limit viral replication, while other cytokines, including IL-6, TNF-α and IL-1β, recruit immune cells to the site of infection and elicit inflammation. NF-κB-dependent and IRF-dependent cytokines are transcriptionally regulated, whereas inflammasome-dependent IL-1β secretion is regulated both transcriptionally and post-transcriptionally (Figure 1). Importantly, many viruses can suppress these innate responses at the 'sensing' and/or transcriptional level upon replication within infected cells [14].

Immune recognition of viruses by transmembrane innate receptors

Transmembrane innate receptors, such as TLRs, recognize extracellular viruses, and their activation does not

Figure 1



Innate immune receptors involved in virus recognition. **NLR and AIM2 pathways**: NLRP3 is activated by a wide variety of stimuli, including RNA viruses. Foreign cytoplasmic dsDNA is also detected by AIM2 via the HIN200 domain. Their activation induces the recruitment of the adaptor protein, ASC, via the pyrin domain. Procaspase-1 is also recruited to ASC via the CARD domain (inflammasome formation). This interaction leads to the auto-cleavage of caspase-1 and results in the activation of caspase-1, which cleaves pro-IL-1β. NOD2 is involved in the recognition of ssRNA viruses. NOD2 activates IPS-1, a mitochondrial membrane-anchored protein, through the NBD and LRR domains, which leads to IRF3 activation. **RLR pathway**: RIG-I is essential for IFN responses to several ssRNA viruses such as *Orthomyxoviridae* and *Paramyxoviridae*. However, MDA5 is necessary for responses to a different set of viruses, such as *Picornaviridae*. LGP2 can act as a positive regulator, making viral RNP complexes more accessible to RIG-I and MDA5. Some viral DNAs are transcribed into 5′ tri-phosphate RNA (the RIG-I ligand) by cytosolic RNA polymerase III (pol III). RIG-I and MDA5 signal via the adaptor protein, IPS-1, which leads to type I IFN production through the TBK1-IRF3-dependent pathway, and proinflammatory cytokine production through NF-κB translocation. RIG-I can activate the inflammasome by interacting with the CARD domains of RIG-I and ASC, and produce IL-1β. **Cytosolic DNA sensor pathways**: extra-chromosomal histone H2B binds DNA virus-like HPV through its α-helical region and interacts with IPS-1 via association with the adaptor protein CIAO. IFI16 binds DNA viruses via the HIN200 domains. They then activate the STING-TBK1-IRF3-dependent signaling pathway, resulting in the production of type I IFN. DAI detects DNA viruses and induces TBK1-IRF3-dependent type I IFN production. LRRFIP1 detects both bacterial DNA and viral RNA from VSV and induces type I IFN production via the β-catenin-IRF3 transactivator pathway. The DExD/H box helicase, DHX9/36, detects CpG-ODNs and DNA viruses such as HSV, leading to MyD88-IRF7-dependent type I IFN production. **TLR pathway**: some RNA viruses are detected by cell surface TLR2 and TLR4, which induce MyD88-dependent NF-κB activation. TLR4 is also recruited to the endosome, leading to TRIF-dependent type I IFN production. TLR3 and TLR7/8 recognize dsRNA and ssRNA, respectively, from RNA viruses. TLR3 induces TRIF-TBK1-dependent type I IFN production, whereas TLR7/8 induces NF-κB and IRF7 activation via MyD88. TLR9 detects CpG-ODNs and DNAs derived from DNA viruses, leading to NF-κB and IRF7 activation via MyD88. Some DNA viruses are also recognized by TLR2 in the endosome, which then induces IRF3/7-dependent type I IFN production.

necessarily require infection of the receptor-expressing cells. Based on cellular localization, TLRs can be grouped in two types: cell surface TLRs (TLR1,2,4,5,6) and endosomal TLRs (TLR3,7,8,9) [1]. Cell surface TLRs recognize bacterial/fungal cell wall components. However, many reports show that some viral proteins are also recognized by cell surface TLR2 and TLR4 [15,16]. A recent report by Barbalat *et al.* identified another interesting example of viral recognition by cell surface TLRs. Mouse cytomegalovirus and vaccinia virus (both dsDNA viruses) were recognized via TLR2. This led to the production of IFN-β, which was not observed upon stimulation with Pam3SK4 (a well-known bacterial TLR2 agonist) [17••]. Interestingly, this TLR2-mediated IFN-β production was restricted in Ly6C(hi) inflammatory monocytes, and was dependent on TLR2 recruitment from the cell surface to the endosome [17••]. However, the exact molecular mechanism(s) underlying virus recognition by cell surface TLRs is the subject of future research. The endosomal TLRs, TLR3, TLR7/8, and TLR9 recognize virus-derived dsRNA, ssRNA, and DNA, respectively [18]. Many viruses are recognized by these endosomal TLRs (Figure 1). TLR3 signaling is mediated by the adaptor molecule TRIF, which induces IRF3 phosphorylation leading to IFN-β production. TLR7/8/9 signaling is mediated by another adaptor molecule, MyD88 (an adaptor commonly used by other TLRs, except TLR3) leading to IRF7-mediated IFN-α production. Importantly, expression of these endosomal TLRs is restricted to certain types of dendritic cells (DCs). TLR3 is preferentially expressed by CD8α(+)DCs, and TLR7/9 is preferentially expressed by plasmacytoid DCs (pDCs). Overall, the recognition of the presence of viruses seems to be mediated by limited types of host cells that express these transmembrane innate immune receptors.

Immune recognition of viruses by cytosolic innate receptors

In contrast to transmembrane receptors, cytosolic innate receptors are expressed by all host cells. RLRs and NLRs mainly recognize viral RNAs, and the recently identified ALRs (and other cytosolic DNA sensors) detect viral DNA in the cytosol of infected cells. This cytosolic receptor-mediated virus recognition is critically important for the host innate immune responses to contain viral replication within the infected cells before the adaptive immune responses are fully developed. In contrast, the contribution of this form of cytosolic virus recognition to adaptive responses is varied and more controversial, as discussed later in this review.

RLRs comprise retinoic acid inducible gene-I (RIG-I), melanoma differentiation associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2). Both RIG-I and MDA5 recognize viral RNAs within the cytoplasm of infected cells. However, the exact molecular signatures of the RIG-I and MDA5 ligands are still not fully understood [19–22]. Owing to the lack of a caspase recruitment domain (CARD), which is important for interactions with IPS-1, LGP2 was assumed to function as a negative regulator of RIG-I and MDA5. However, a recent study suggests that LGP2 positively regulates RIG-I and MDA5 signaling, possibly by modifying the viral RNA structure before detection by these two receptors [23]. Virus recognition by RIG-I and MDA5 is mediated by a single adaptor molecule, IPS-1 (or MAVS), and leads to NF-κB and IRF3/IRF7 activation. Interestingly, a recent report demonstrated that RIG-I can directly activate ASC in an NLRP3-independent manner, leading to caspase-1-dependent IL-1β production during VSV (ssRNA virus) infection [24••].

NLRs comprise a large number of family member proteins that contain a conserved NOD motif [25], and can be classified into two groups. Activation of Nod1 and Nod2 leads to the activation of NF-κB and IRF. Although Nod2 was initially characterized as a cytosolic sensor for the bacterial cell wall component, muramyl dipeptide, which induces NF-κB activation, a recent report suggests that Nod2 also functions as a virus sensor [26••] and activates a non-classical NLR signaling pathway [27]. Sabbah *et al.* showed that Nod2 can directly sense cytosolic ssRNA from RSV and influenza virus, leading to MAVS(IPS-1)-IRF3-mediated IFN-β responses [26••]. Activation of NLRs, such as NLRP1, NLRP3, and NLRC4, leads to inflammasome formation, which results in caspase-1-mediated IL-1β and IL-18 secretion (Figure 1). The NLRP3 inflammasome is one of the best characterized inflammasomes, and is activated by bacterial toxins, LPS, and viral RNAs, as well as uric acid and alum [11•,28]. Interestingly, it appears that many RLRs and NLRs sense virus infections by detecting viral genomic, or replication-intermediate, RNA. This might indicate that the presence of viral nucleic acids provides the stronger proof of active viral infection, rather than general danger signals.

Cytosolic DNA sensors

AIM2 and IFI16 are both recently identified cytosolic DNA sensors and are involved in DNA-dependent inflammasome activation and IFN-β production, respectively [5,29–34]. Because both proteins contain a PYHIN domain [35–37], it has been proposed that they be referred to as ALRs [5]. However, several other molecules are also known to be involved in DNA sensing within the cytosol. DAI (ZBP-1) is the first reported DNA sensor molecule that triggers TBK1-IRF3-dependent IFN-β induction *in vitro* [38]; however, gene knockout mice do not show the same phenotype, suggesting the presence of redundant DNA sensor mechanisms [39]. Lrrfip1 recognizes cytosolic dsDNA (and dsRNA), subsequently interacting with β-catenin and enhancing IRF3-mediated IFN-β expression [40]. DHX36 and DHX9, present in human pDC, are cytosolic CpG-A and CpG-B binding

proteins, respectively. These proteins mediate the MyD88/IRF7-dependent production of IFN-α [41]. AT-rich DNA is also recognized indirectly by RNA polymerase III. AT-rich DNA is transcribed into 5-triphosphate dsRNA, which is then recognized by the RIG-I pathway [42,43]. The cytosolic histone, H2B, is also involved in DNA sensing. The dsDNA/H2B complex activates IPS-1 via CIAO (an adaptor molecule that links histone H2B and IPS-1) in human cell lines leading to IFN-β expression. However, this H2B-mediated dsDNA-dependent IFN-β production is not observed in mice, most likely because of the lack of the interaction between mouse CIAO and mouse IPS-1 [44].

Signaling via which innate immune receptors leads to adaptive immune responses: TLRs, RLRs, NLRs, or others?

Activation of the innate immune system is critical for establishing adaptive immune responses. This is simply demonstrated by the fact that immunization with a highly purified recombinant protein is usually unsuccessful owing to the lack of innate responses [45]. On the other hand, viral infections are usually sensed by multiple innate receptors. The live attenuated yellow fever vaccine 17D has been shown to activate multiple TLRs, resulted in CD8T and a mixed Th1/Th2 immune responses [46,47]. In the case of influenza virus, infection can be detected by three different receptors: TLR7, RIG-I, and NLRP3 [48•,49]. However, conflicting results have been reported, particularly in terms of the adaptive immune responses examined in these receptors/adaptors deficient mice (Table 1).

The involvement of TLR7/MyD88 has been examined by four independent studies [50–53]. It is very difficult to generalize the results, which range from almost nothing to identifying a prominent phenotype, except that all of the studies consistently agreed that CD8T responses were not affected by the absence of the TLR7/MyD88 pathway. However, two independent studies consistently demonstrated that, in contrast to live virus, the immunogenicity of a chemically killed (inactivated) whole virus was completely dependent on TLR7/MyD88 signaling [48•,54•].

The RIG-I/IPS-1 pathway was also examined in two independent studies [52,53]. They concluded that although RIG-I/IPS-1 signaling induces almost overlapping cytokine responses to those induced by TLR7/MyD88 (Figure 1), IPS-1-deficiency had no substantial effect upon adaptive responses to influenza virus infection [52,53]. This may reflect differential cellular expression of these receptors. RIG-I is ubiquitously expressed by most cells, whereas TLR7 is preferentially expressed by pDCs. It may also reflect the fact that RIG-I sensing requires viral replication within the cell, whereas TLR7 recognizes viruses in the endosome, which is not dependent upon virus infection (Figure 1). Differential regulation of adaptive immune responses by TLRs and RLRs has also been reported in another virus infection system. Jung *et al.* demonstrated that during LCMV infection, CD8T responses in MyD88-deficient mice were significantly reduced, whereas IPS-1-deficient mice showed comparable CD8T responses to those of wild-type mice [55].

NLRP3 can be triggered by viral RNA [56] and/or ionic perturbation caused by the influenza M2 protein [57]. NLRP3 triggers ASC-mediated NLRP3 inflammasome formation, leading to caspase-1-dependent IL-1β and IL-18 secretion. Inflammasome involvement in influenza virus infection has been studied by four independent

Table 1

Adaptive immune responses in mice deficient in innate immune receptors/adaptors against influenza virus infection and vaccination.

	Virus	TLRs(Myd88) deficiency	RLRs(IPS-1) deficiency	NLRs(NLRP3, ASC, caspase-1) deficiency
		IFN-I, TNFα/IL-6		Inflammasome (IL-1/IL-18)
Lopez *et al.* [50]	A/PR8	CD4(IFNγ)→, CD4(IL-4)T, CD8→, Ab→	Not examined	Not examined
Heer *et al.* [51]	A/PR8	CD4→, CD8→, Ab(IgG2a)↓	Not examined	Not examined
Koyama *et al.* [52]	A/PR8, A/NC	CD4(IFNγ)↓, CD8→, Ab(IgG2a)↓	CD4(IFNγ)→, CD8→, Ab→	Not examined
Seo *et al.* [53]	A/PR8	CD4(Th1)↓, CD4(Th2)↑, CD8→, Ab→	CD4(Th1)→, CD8→, Ab→	Not examined
Ichinohe *et al.* [58]	A/PR8	Not examined	Not examined	CD4(IFNγ)↓ CD8↓, Ab(IgG, IgA)↓
Allen *et al.* [59]	A/PR8	Not examined	Not examined	Intact adaptive responses (CD8→, Ab→)
Thomas *et al.* [60]	A/PR8	Not examined	Not examined	CD8→, Ab→
Koyama *et al.* [48•]	A/NC	Not examined	Not examined	CD4(IFNγ)→, CD8→, Ab(IgG1)↓
	Inactivated WV(A/NC)	CD4→, Ab↓	CD4→, Ab→	CD4→, Ab→
Geeraedts *et al.* [54•]	Inactivated WV(H5N1)	CD4↓, Ab↓	Not examined	Not examined

groups. Ichinohe *et al.* demonstrated that NLRP3-independent, but ASC-dependent, inflammasome responses were important for both CD4T and CD8T responses, as well as IgA and IgG responses [58] (it is noteworthy that Poeck *et al.* also reported NLRP3-independent, but RIG-I and ASC-dependent, inflammasome activation by VSV [24••]). In contrast, Allen *et al.* and Thomas *et al.* showed that NLRP3 inflammasome responses were not involved in adaptive responses, but play a more important role in the innate phase of host defense and in tissue healing [59,60]. We also examined ASC-deficient mice and found that inflammasome activation had almost no impact on the adaptive response to live influenza virus infection [48•]. At present, the reason for these contradictory results is not clear [11•,61].

Viral subversion of innate immune responses may affect adaptive immune responses

These controversies may be explained by differences in the types of virus used; especially the different subversion mechanisms used by the viruses. Influenza virus (and other viruses) possesses an immune evasion protein that modulates the innate immune signaling cascades of the host [14]. Even though most studies used a mouse-adapted PR8 virus, Heynisch *et al.* reported that two variants of A/PuertoRico/8/34 show very different activation patterns for cellular signaling molecules in MDCK cells [62]. This most likely reflects the fact that these variant viruses modulate cytosolic signaling systems in different ways. Influenza NS-1 is the most well-characterized of the proteins that subvert RIG-I mediated IFN-α/β responses at multiple steps [63]. A recent report suggests that the inflammasome is also an evasion target of a herpes virus [64]. Intriguingly, no direct viral mechanism that antagonizes TLR signaling has been described for influenza A virus [63]. Taken together, these data suggest that the same PR8 virus may induce very different host immune responses. Furthermore, they may also suggest that subverting the infection-dependent cytosolic innate system may be easier than subverting the infection-independent TLR system. In line with this hypothesis, once the virus is fixed with formalin (and killed), the host immune response is consistently TLR7/MyD88-dependent [48•,54•].

Conclusions

The existence of diverse innate immune receptors may reflect a redundancy that ensures sensitive detection of viruses in a variety of tissue and cell types, and the subsequent induction of host defense mechanisms. TLRs can detect extracellular viruses (either live or dead), and do not require viral infection of receptor-expressing cells. By contrast, detection by cytosolic receptors requires viral infection and replication, which can be easier evasion targets for many viruses. The innate immune response plays two roles in host defense: (1) it limits (or at least controls) viral replication during initial infection; and (2) it induces adaptive immune responses responsible for viral clearance and maintenance (memory). However, it is still not clear to what extent each innate immune receptor contributes to the adaptive immune responses. Owing to sophisticated immune evasion mechanisms, infection by live viruses may not provide a clear answer. However, immunization with an inactivated whole virion influenza vaccine clearly demonstrates that TLR-mediated innate signaling alone is sufficient to induce adaptive immune responses. Currently, it is difficult to examine the individual contribution of each RLR and NLR to the adaptive immune response because of the lack of selective activators. Recently, Kasturi *et al.* demonstrated that synthetic nanoparticle based vaccines composed of multiple TLR ligands induced persistent antibody and CD8T responses than single TLR activating vaccine [65]. It suggests that activations of multiple innate immune receptors may be required for long lasting memory responses but not necessarily required for mounting temporal effector responses. Further studies will clarify the more detailed coordination between innate and adaptive immune responses, and provide a more rational way of vaccine design.

Conflicts of interest statement

The authors have no conflicts of interest to declare.

Acknowledgements

The authors thank Drs. Cevayir Coban and Fumihiko Takeshita for helpful discussion, and all members of Ishii's and Coban's laboratory. This work was supported by Ministry of Health, Labour and Welfare (MHLW) (KJI), the Knowledge Cluster Initiative (KJI), a Grant-in-Aid for Scientific Research (KAKENHI) (TA, KK and KJI) from the Japanese Ministry of Education, Culture, Sports, Science and Technology, and by CREST, JST (KJI).

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