Review

Innate antiviral signalling in the central nervous system

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The innate immune system mediates protection against neurotropic viruses capable of infecting the central nervous system (CNS). Neurotropic viruses include herpes simplex virus (HSV), West Nile virus (WNV), rabies virus, La Crosse virus, and poliovirus. Viral infection triggers activation of pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs), retinoic acid-inducible gene 1 (RIG-I) like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and cytosolic DNA sensors. Although originally characterised in peripheral immune cells, emerging evidence points to important roles for these PRRs in cells of the CNS. Here, we review recent advances in our understanding of the mechanisms by which these PRRs provide protection against neurotropic viruses, and discuss instances in which these responses become detrimental and cause immunopathology in the CNS.

PRRs control antiviral immunity

The CNS occupies a pivotal role in living organisms associated with cognition and higher-order functions and is key to their successful survival and propagation. Similar to many other organs, the CNS is susceptible to infection by invading microorganisms including viruses. Therefore, it is not surprising that mechanisms exist in the CNS to defend against such infections. The innate immune system consists of a network of PRRs that detect conserved pathogen-associated molecular patterns (PAMPs) of microbes [1]. These PRRs activate the transcription factors nuclear factor (NF)-κB and interferon (IFN) regulatory factor (IRF) family members such as IRF3. This results in the activation of mechanisms of direct intrinsic immunity, which include inhibition of protein synthesis, and also in the secretion of effector cytokines, chemokines and type 1 IFNs (IFNα and IFNβ) (Figure 1). In the context of antiviral immunity, specific PRRs are activated by nucleic acids, the dominant PAMPs of viral infection. This initial host response to infection also triggers and shapes the ensuing adaptive immune response [2]. Although best characterised in the periphery, there is a growing understanding of the innate mechanisms of antiviral immunity that function in the CNS.

Type I IFNs in particular are powerful antiviral mediators and establish an 'antiviral state' in infected and adjacent cells. Mechanistically, this occurs by the binding of IFNα and IFNβ to the IFN-α/β receptor (IFNAR), leading to the expression of IFN response genes (ISGs). These ISGs, numbering more than 300 [3], have a wide range of antiviral activity and include ISG49, ISG54, and ISG56, which are expressed in the CNS after viral infection [79].

Four main classes of PRRs have been reported: TLRs, RLRs, NLRs, and cytosolic DNA sensors [5]. Many of these receptors and their associated intracellular signalling molecules are expressed in cells of the CNS [6], and so might be expected to respond to the wide range of viruses capable of infecting the CNS, including HSV, WNV, rabies virus, and poliovirus. Here, we review recent findings that provide new insights into how the innate immune system of the CNS, and PRRs in particular, provide immunity to such viruses. We also discuss how viral activation of innate immunity in the CNS can, in some instances, lead to the overproduction of inflammatory mediators resulting in virally induced neuropathology.

Viral sensing by TLRs in the CNS

TLRs are the best studied PRRs, of which, ten are functional in humans and 12 in mice [7]. TLRs can be broadly grouped into those that are expressed on the cell surface and detect PAMPs of mainly bacterial origin, and those that are expressed intracellularly in endosomes and detect viral nucleic acids [8]. The latter include a receptor that detects double-stranded RNA (dsRNA) (TLR3), two that sense single-stranded RNA (ssRNA) (TLR7 and TLR8), and one that responds to undermethylated (CpG) double-stranded DNA (dsDNA) (TLR9). Signalling downstream of TLRs relies on the recruitment of the Toll-interleukin receptor (TIR) adaptor proteins myeloid differentiation primary response 88 (MyD88), MyD88 adaptor like/toll-interleukin 1 receptor (TIR) domain-containing adaptor protein (Mal/TIRAP), Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF) and TRIF-related adaptor molecule (TRAM) [9]. MyD88, the prototypical member of the TIR group, is utilised by all TLRs with the exception of TLR3, which uses TRIF. Engagement of TLRs triggers TIR adaptor recruitment to activate IκB kinases (IKK)s such as TANK binding kinase 1 (TBK1) and IKKβ culminating in the activation of NF-κB and IRF

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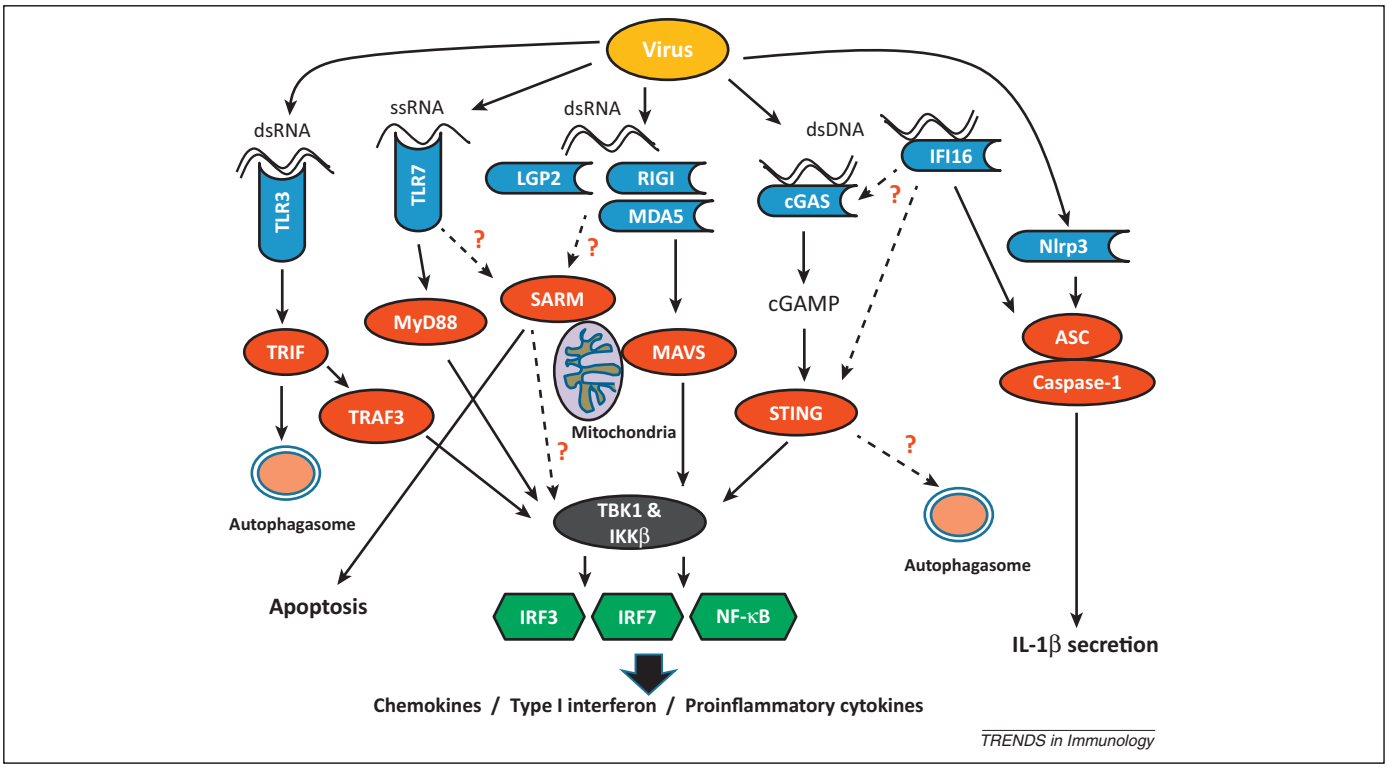

Figure 1. Antiviral signalling pathways in the central nervous system. Viruses can be detected by one of four classes of pattern recognition receptors (PRRs) in the central nervous system (CNS): Toll-like receptors (TLRs), retinoic acid-inducible gene (RIG)-like receptors (RLRs), the NLR family, pyrin domain-containing protein 3 (Nlrp3) inflammasome, or DNA sensors. Engagement of TLRs results in activation of myeloid differentiation primary response 88 (MyD88)- and Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF)-dependent signalling pathways to activate proinflammatory cytokines and type I interferons (IFNs). TLR activation of TRIF signalling can also trigger autophagy. RLR activation by viruses triggers mitochondrial antiviral-signaling protein (MAVS)-dependent signalling to activate the transcription factors interferon (IFN) regulatory factor (IRF)3, IRF7, and nuclear factor (NF)-κB to trigger inflammatory gene induction. Sterile alpha and TIR motif-containing protein (SARM), which is also located at the mitochondria and activated by viruses, can induce cytokines and chemokines by currently unknown mechanisms. SARM can also trigger apoptosis in response to specific viruses. Cytosolic DNA from viruses binds and activates the enzyme cyclic GMP-AMP synthase (cGAS) leading to the production of cyclic guanosine monophosphate-adenosine monophosphate (cGAMP). This second messenger activates stimulator of interferon genes (STING) and associated signalling pathways that lead to the induction of genes that are also downstream of TLR and RLR signalling. Interferon gamma-inducible protein 16 (IFI16) also senses DNA viruses and stimulates both STING-dependent IFN induction and caspase 1 activation. Whether IFI16 acts via cGAS for IFN induction is currently unclear. At present, the pathway activated by cytosolic DNA to trigger autophagy is also unknown. Finally viral infection can activate the Nlrp3 inflammasome, the cytosolic caspase-1 containing platform to trigger interleukin (IL)-1β secretion.

family members (Figure 1) [9]. This results in the expression of genes that lead to pathogen elimination.

TLRs are widely expressed in the CNS in both the mouse and human systems [10] and have been shown to respond to neurotropic viruses. Table 1 provides a generic overview of the principal steps of viral pathogenesis in the CNS and describes the role of the PRRs in the innate immune response to the virus at each step. Given the propensity of inflammatory responses to cause tissue damage, some TLR-stimulated responses to viruses in the CNS are detrimental (Table 2). Our understanding of TLR responses to both RNA and DNA viruses in the CNS has increased in recent years, as discussed below.

TLRs provide protective immunity against CNS viruses

Poliovirus, an ssRNA virus of the Picornaviridae family, causes paralysis upon CNS entry. TLR3 confers protective immunity to infection by poliovirus; the TLR3–TRIF signalling pathway was demonstrated to limit viral replication in many organs including the brain and spinal cord [11]. The recently characterised endosomal TLR, TLR13, is expressed in mice but not humans and requires MyD88 for signalling. This TLR was shown to sense vesicular stomatitis virus (VSV) [12]. VSV is a neurotropic ssRNA virus and a member of the Rhabdoviridae family. It is a zoonotic virus that causes a disease similar to foot and mouth disease in animals and influenza-like illness in humans. The importance of TLR signalling in the response to VSV is further highlighted by the finding that mice lacking MyD88 exhibit reduced survival with increased viral load in the CNS [13]. WNV, a mosquito born flavivirus, can cause encephalitis and meningitis in infected individuals. Studies on the role of TLRs in response to WNV have mainly focused on TLR3 and TLR7, and have presented contradictory results. In the case of TLR3, one study reported a positive role for TLR3 in mediating antiviral immunity [14], whereas another showed that TLR3 contributes to WNV pathogenesis [15]. A similar situation exists for TLR7, where one study demonstrated a protective role for TLR7 [16], whereas another showed that TLR7 may actually contribute to viral dissemination [17]. Nevertheless, it is likely that TLR responses are important for protective immunity against WNV, because mice lacking MyD88 show reduced survival in response to this virus [16,18]. In addition, recent work has shown that the TLR7–MyD88 axis is necessary for induction of an adaptive immune response to an attenuated form of the virus [19]. Thus, although the discrepancies in previous reports remain to be reconciled, TLRs overall likely play a protective role against WNV infection.

Table 1. Principal steps in viral pathogenesis in the CNS.

Steps in viral pathogenesis	Cells/tissue/process	Role for the innate immune system	Refs
1. Entry into the CNS	Blood brain barrier (BBB)	TLR3 stimulates inflammatory responses contributing to break-down of the BBB;	[15]
2. Viral replication	Neurons	Autophagy restricts viral replication; IFN-stimulating pathways like the UNC93B–TLR3 and RLR pathways restrict viral replication; the Nlrp3 inflammasome pathway synergises with the IFN pathway to restrict viral replication	[90] [25] [28] [52] [14] [58]
	Astrocytes	The TLR3 pathway stimulates IFN expression and restricts viral replication	[27]
	Oligodendrocytes	The UNC93B–TLR3 pathway stimulates IFN expression and restricts viral replication	[29]
3. Spread within the CNS		TLR3-driven IFN expression in astrocytes prevents viral spread beyond the site of entry and productive infection of non-neurons; the RLR–MAVS pathways prevents dissemination of virus from the site of entry into the CNS	[27] [28] [52]
4. Tissue damage	Microglial cells, infiltrating macrophages	TLR2-dependent inflammatory reactions; the Nlrp3 inflammasome pathway contributes to neuroinflammation	[91] [61]
	$CD8^+$ T cells	MDA5 shapes $CD8^+$ T cell activation for optimal clearance of virus from the CNS	[92]
	Cytopathic effect	Defective innate antiviral immunity facilitates cytopathic replication; TLR3 is involved in formation of inclusion bodies	[4]

Much research has focused on the immune response to HSV in the CNS, because HSV infection is a major cause of herpes simplex encephalitis (HSE). The pathology of HSE involves both viral cytopathic effects and immunopathology [20]. Work from the Casanova group has led to identification of several single-gene mutations in the TLR3 pathway in children with susceptibility to HSE, hence strongly indicating this pathway as being essential for protective responses. TLR3 signals via TRIF, TRAF3, and TBK1 to induce type I IFNs. Currently, mutations in TLR3, TRIF, TRAF3, and TBK1 as well as in uncoordinated-93B (UNC-93B), a chaperone essential for endosomal TLR sorting, have been found in children with HSE [21–25] (Table 2). In 2011, the Casanova group reported a patient with complete TLR3 deficiency [26]. Similar to previous studies, the only susceptibility experienced by this patient was to HSE. Antiviral functions were normal in TLR3-deficient leukocytes, indicating that in the periphery, TLR3 functions can be compensated by other innate immune sensors. Thus, the sole demonstrated nonredundant function of TLR3 in humans is protection from infection by HSV and the ensuing childhood HSE.

Although *in vitro* studies have demonstrated that HSV can be detected by TLR2, TLR3, and TLR9, as well as by intracellular RNA and DNA sensors such as RIGI and interferon gamma-inducible protein 16 (IFI16) [20] (discussed below), it is striking how the TLR3-driven response seems to dominate. Therefore, outstanding questions in the field are what are the underlying mechanisms whereby the TLR3 pathway defends against HSV, and how these differ from those triggered by other PRRs that are capable of recognising HSV, based on *in vitro* studies. Recent studies in mouse models have demonstrated essential roles for TLR3 and TRIF in virus control in the CNS. In these studies, CNS infection by HSV was measured following vaginal or intranasal inoculation of mice lacking TLR3 or TRIF [27,28]. Reinert *et al.*, studying HSV-2 infection, revealed that, in their model system, TLR3 acted to prevent viral entry and spread within the CNS. The virus gained broader tropism in TLR3-deficient mice, with infection notably reaching astrocytes [27]. The Notarangelo group recently used a model wherein induced pluripotent stem cells from the dermal fibroblasts of TLR3- and UNC-93B-deficient patients were differentiated into populations of neural stem cells, neurons, astrocytes, and oligodendrocytes [29]. The authors found that UNC-93B-deficient neurons and oligodendrocytes exhibited an impaired IFN response and increased viral loads. Together with the murine studies cited above, these data point to a role for TLR3 in antiviral defence in a broad range of cell types in the CNS.

HSV is a DNA virus and TLR3 is a sensor for dsRNA, therefore, it is not clear how TLR3 senses HSV infection. Previous studies have demonstrated that dsRNA accumulates in the cytoplasm of cells productively infected with HSV-1 [30]. Alternatively, dsRNA produced by infected cells may in fact be sensed by TLR3 in adjacent immune cells phagocytosing dying cells [31]. In addition, the antiviral effector mechanisms downstream of TLR3 in the CNS require clarification. Although the common belief is that antiviral immunity in the CNS proceeds largely through induction of type I IFN, autophagy is also likely involved. Autophagy is used during cellular stress in order to regenerate metabolic precursors and clear subcellular debris, and it is now appreciated also to have a role in immunity to pathogens (Box 1). It is possible that TLR3-triggered autophagy plays a role in protection against HSV-1 in the CNS, because TLRs are known to stimulate autophagy through a pathway involving TRIF [32]. In light of this, it is interesting to note that the HSV-1 protein ICP34.5 targets beclin-1, a protein essential to autophagy, and that the functions of ICP34.5 as a neurovirulence factor are dependent on its ability to target beclin-1 [33]. Although further research will be required to define the mechanisms underlying TLR3 functions, the aforementioned studies highlight a protective role for the TLR3 signalling pathway in defending against HSV in the CNS.

TLR-associated neuropathology in response to some CNS viruses

There are several examples where the immune response to a neurotropic RNA virus contributes to its pathogenesis (Table 2). One such example is infection with rabies virus, an ssRNA virus of the Rhabdoviridae family. Control of rabies

Table 2. PRRs and signalling proteins involved in protection or pathological response to neurotropic viruses.

Virus	Viral genome	PRR or signalling protein tested	Neuroprotection (model system used)	Neuropathology (model system used)	Refs
HSV-1	DNA	TLR3	Yes (human)		[21]
		TRIF	Yes (human)		[22]
		TRAF3	Yes (human)		[23]
		TBK1	Yes (human)		[24]
		UNC-93B	Yes (human)		[25]
		TLR3	Yes (mouse)		[27]
		TRIF	Yes (mouse)		[28]
		MAVS	Yes (mouse)		[28]
		TLR3	Yes (human iPCs)[a]		[29]
		STING	Yes (mouse)		[69]
VSV	RNA	MyD88	Yes (mouse)		[13]
		SARM		Yes (mouse)	[41]
		IFIT2	Yes (mouse)		[79]
Rabies	RNA	TLR3		Yes (mouse)	[4]
		MAVS	Yes (mouse)		[48]
		LGP2	Yes (mouse)		[35]
West Nile virus	RNA	TLR7/MyD88	Yes (mouse)		[19]
		SARM	Yes (mouse)		[43]
		MAVS	Yes (mouse)		[52]
		NALP3[a]	Yes (mouse)		[58]
		Caspase-1	Yes (mouse)		[58]
		IL-1R	Yes (mouse)		[58]
		ASC	Yes (mouse)		[60]
Sindbis virus	RNA	MAVS	Yes (mouse)		[54]
La Crosse virus	RNA	SARM		Yes (mouse)	[42]
Poliovirus	RNA	TLR3, TRIF	Yes (mouse)		[11]

[a]iPC, induced pluripotent stem cell; NALP, NLR family, pyrin domain-containing protein.

virus infection depends on the induction of a rapid host antibody response, which is usually insufficient in infected individuals to prevent viral entry into peripheral nerves [34]. The virus then migrates to the CNS where its immunosuppressive properties lead to rapid viral replication in neurons resulting in fatal encephalitis [34,35]. In an intriguing study by Menager and colleagues, it was shown that TLR3 was necessary for the formation in human neuronal cells of Negri bodies – perinuclear inclusion bodies consisting of TLR3 surrounded by viral proteins and viral genomic material [4]. Mice lacking TLR3 had reduced viral load and improved survival in response to the virus [4], suggesting that TLR3 contributes to rabies virus pathogenesis.

Altogether, these studies have shown that there are virus-specific outcomes to TLR-based signalling during viral infections in the CNS, which may either benefit the host or the virus.

The TIR adaptor SARM mediates protection and pathologies in response to CNS viruses

Sterile alpha and TIR motif-containing protein (SARM) is the fifth member of the TIR adaptor protein family that also comprises MyD88, Mal/TIRAP, TRIF, and TRAM. SARM is the most highly conserved TIR adaptor protein [9]; it is the only mammalian TIR adaptor protein with an ortholog present in *Caenorhabditis elegans* [36]. This protein, known as TIR-1, has functions both in *C. elegans* development and immunity [37,38]. Human SARM was initially shown to be an inhibitor of TRIF-dependent TLR signalling [39]. In mice, SARM is expressed mainly in the CNS and has been shown to mediate neuronal death in response to oxygen and glucose deprivation [40]. In the CNS, SARM appears to have TLR-independent functions even though it contains a TIR domain [40]. SARM has been reported to contribute to the proinflammatory response to VSV in the CNS; being required for chemokine and type I IFN gene induction following VSV infection [41]. Exactly how SARM mediates gene induction is currently unknown, because SARM fails to activate IRF3 or NF-κB [39] (Figure 1). SARM mediates cytokine production by neurons in response to VSV, and this process was shown to be dependent on the presence of microglia, indicating an important role for cell communication in the antiviral functions of SARM in the CNS [41]. Interestingly, mice lacking SARM show reduced inflammation and improved survival in response to the virus [41]. Therefore, SARM in this case can be regarded as a mediator of immunopathology during VSV infection where the absence of SARM reduces the inflammatory response to the virus, thus improving survival (Table 2).

It was recently reported that SARM mediates apoptosis in neurons in response to La Crosse virus [42], a member of the bunyavirus family and a leading cause of paediatric encephalitis. In neurons SARM localises to mitochondria, binds ATP synthase following viral infection, and leads to the production of reactive oxygen species (ROS), resulting in oxidative stress and apoptosis [42]. Mukherjee *et al.* reported that mice lacking SARM exhibited less neuronal damage upon viral infection. The authors propose that inhibition of SARM may provide clinical benefit during La Crosse virus infection. A requirement for SARM for immunity to WNV has also been shown; its importance in this context being associated with TNF expression [43].

Box 1. Autophagy and antiviral immunity

Autophagy is a homeostatic process that takes place in all eukaryotic cells whereby cytoplasmic components are surrounded by a double membrane to form autophagosomes [93]. These then fuse with lysosomes, to form an autolysosome, leading to degradation and recycling of the vesicle contents. Autophagy initiates in response to environmental cues such as energy depletion and starvation, and it can be inhibited by insulin and growth factor signalling. Autophagy is a survival mechanism used in response to cellular stress so as to regenerate metabolic precursors and clear subcellular debris [93]. However in recent years autophagy has also been linked to innate and adaptive immune responses to invading pathogens. Many studies that have linked autophagy with immune responses have relied on loss of function experiments using cells or mice deficient in autophagy-related genes (*Atgs*), or RNAi to knockdown ATG protein expression. Such studies have shown that autophagy can directly clear intracellular pathogens, for example, by engulfing and degrading virions [78]. Autophagy can also enhance antigen presentation by translocating endogenous antigens from the cytosol to MHC class I and II complexes, and can promote IFN responses by delivering viral PAMPs to endosomal TLRs [78]. In fact, genes essential for autophagy have been shown to be protective *in vivo* in the case of some intracellular pathogens. Furthermore, human genome-wide association studies have also identified associations between single nucleotide polymorphisms (SNPs) in genes known to regulate autophagy and susceptibility to inflammatory and autoimmune disease [93]. For example, several SNPs in ATG5, which is an essential part of the Atg5–Atg12–Atg16 conjugation system required for autophagosome formation, have been linked to lupus susceptibility. Successful intracellular pathogens have also been shown to have mechanisms to evade and subvert the autophagy pathway. Some proteins known to have a role in regulating autophagy have been shown to also regulate inflammatory and immune responses. For example, autophagy-related gene 9a (Atg9a) was shown to regulate the innate immune response to cytosolic DNA by interacting with STING, and loss of Atg9a enhanced recruitment of the kinase TBK1 to STING, and of STING-dependent cytokine and IFN induction [94].

Mice lacking SARM exhibited increased WNV replication in the brainstem and overall reduced survival rates, highlighting a positive role for SARM in the effective resolution of WNV infection [43] (Table 2).

In summary, the innate immune response to several CNS viruses is dependent on SARM, although whether SARM has a protective or pathological role depends on the specific virus. Why SARM mediates apoptosis to La Crosse virus and not to other RNA viruses such as WNV and VSV is unknown, but this might be due to variation in the way different viruses induce SARM expression. It is possible that viruses that induce high levels of SARM expression trigger apoptosis. The next critical steps in understanding SARM are to determine the mechanism whereby SARM is activated to trigger apoptosis, and how SARM can trigger inflammatory gene induction, because it does not function like the other TIR adaptor proteins [39]. For example, the mechanism of gene induction may involve ROS production [42]. Intriguingly, two recent reports have demonstrated that SARM has a role in a specific type of neuronal death following mechanical trauma, known as Wallerian degeneration [44,45]. This is regarded as a breakthrough discovery in the field because molecular players in this process were completely unknown. The study by Gerdts and colleagues points to interactions of the sterile α motifs (SAMs) and the TIR domain as being critical to induce this unique mechanism of cell death, but precisely how this process is activated is currently unknown. It will be interesting to determine whether and how this kind of cell death relates to the role of SARM in antiviral responses in the brain. Finally, it is important to note that to date the role of SARM has been examined in response to three RNA viruses in the CNS; it will also be interesting to determine if SARM has any role in mediating innate immune responses to a neurotropic DNA virus, such as HSV.

Emerging roles for RLRs in response to neurotropic viruses

The RLR family consists of RIG-I, melanoma differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2), which signal via the adaptor protein mitochondrial antiviral-signaling protein (MAVS), leading to the activation of NF-κB and IRF family members [46]. Ligands for these cytoplasmic dsRNA sensors have been identified: RIG-I detects blunt-end dsRNA containing a 5′ triphosphate motif, whereas MDA5 detects longer forms of dsRNA such as poly(I:C) [47]. Although less is still known about the role of RLRs in the CNS as compared to TLRs, it has become clear that the RLR pathway is active in the CNS during viral infections.

Type I IFN production and maturation of peripheral bone marrow-derived dendritic cells (BMDCs) in response to rabies virus is dependent upon RIG-I, MDA5, and the adaptor protein MAVS, and is independent of TLR3 and MyD88 [48]. In addition, MAVS-deficient mice show increased limb paralysis upon infection [48], revealing an important role for the RLR pathway in limiting the spread of rabies virus. A recent study has focused on the role of the RLR family member LGP2 in rabies virus infection [35]. Unlike RIGI and MDA5, LGP2 lacks a caspase activation and recruitment domain (CARD) signalling domain [49]. The function of this protein is controversial: early work using overexpression approaches suggests that LGP2 functions to inhibit the RLR pathway [49], but later studies using mice lacking LGP2 have indicated a positive role for LGP2 in the RLR pathway [50]. LGP2 transgenic mice infected with rabies virus exhibited reduced proinflammatory cytokines and type I IFNs as compared to control animals. Unexpectedly, viral titres and morbidity were also reduced [35]. A hallmark of rabies infection is reduced infiltration of T cells in the CNS. This virus also triggers apoptosis in CNS-infiltrating $CD8^+$ T cells, via induction of the immune-inhibitory host protein B7-H1 [35,51]. B7-H1, also known as programmed cell death ligand 1 (PD-L1), acts as an immune regulator by binding to its receptor – programmed cell death 1 (PD1), present on T cells – to inhibit T cell function [51]. It is expressed in lymphoid and nonlymphoid cells such as muscle and endothelia [51]. Crucially, B7-H1 is not normally expressed in neurons, but specifically induced by rabies virus infection [51]. In LGP2 transgenic mice, levels of CNS-infiltrating $CD8^+$ T cells were restored to levels similar to those in control animals, and this was associated with a reduction in B7-H1 expression [35]. Thus, rabies virus has developed an effective means of immune evasion by inducing B7-H1, and the introduction of LGP2, which is not normally expressed in neurons, prevents this and improves outcome [35]. It has been shown that IFNβ is required for rabies virus induction of B7-H1 in neurons [51], therefore, it is likely that LGP2 reduces IFNβ and other type I IFNs during rabies

virus infection. These findings for LGP2, despite the fact that LGP2 is not normally expressed in neurons, reinforce the notion that LGP2 can exert powerful inhibitory effects on the immune system. Although it has not been performed to date, one might predict that analogous studies using either RIG-I or MDA5 might actually contribute to rabies virus pathology.

The role of the RLR pathway in response to WNV infection has also been examined. MAVS was shown to be essential for survival in response to WNV infection; mice lacking MAVS displayed enhanced viral replication and increased susceptibility to WNV [52]. The MAVS pathway was also shown to limit viral replication both in the brain and spinal cord, and also protected neurons; key target cells for WNV. These findings confirm an earlier report on the importance of RIG-I and MDA5 in the innate antiviral response to WNV [53]. Interestingly, mice lacking MAVS displayed increased brain inflammation and a failure in T regulatory (Treg) cell expansion, indicating a dysregulated inflammatory response in the absence of MAVS [52]. This finding may highlight a previously unrecognised regulatory role for MAVS in the resolution of inflammation.

Recently, the MAVS pathway was also reported to limit viral replication in the brain following Sindbis virus infection [54] and in response to the flavivirus Japanese encephalitis virus (JEV) [55]. It is noteworthy that similar to the case for HSV-1, the host response to Sindbis virus infection in the CNS is also dependent on autophagy (Box 1) [56]. Orvedahl *et al*. [56] showed that Sindbis viral capsids were targeted to autophagosomes and that specific inactivation of the autophagy gene *Atg5* in Sindbis-infected neurons resulted in delayed clearance of viral proteins and increased cell death. Although it remains to be determined whether Sindbis-triggered autophagy is RLR-dependent, clearly autophagy protects against both DNA and RNA viruses in the CNS. Of note, MAVS-deficient mice are more susceptible to HSV-1 encephalitis than normal mice are, and display increased viral load and decreased IFNβ production (Table 2); possibly due to RLR-dependent detection of dsRNA, generated during the HSV-1 life cycle [25].

In summary, the RLR system is active to defend against both RNA and DNA neurotropic viruses, and unlike TLRs, all reports to date indicate that RLRs provide protective immunity against these infections and not detrimental responses (Table 2).

The Nlrp3 inflammasome contributes to the innate antiviral response in the CNS

The NLR family, pyrin domain-containing 3 (Nlrp3) inflammasome is a cytoplasmic multi-protein complex consisting of Nlrp3, apoptosis-associated speck-like protein containing a CARD (ASC), and the protease caspase 1. In the periphery, a wide array of cellular insults can stimulate this protein complex to activate caspase 1, including viral RNA [57]. This leads to the cleavage of pro-interleukin (IL)-1β to produce the secretable proinflammatory cytokine IL-1β. This inflammasome system is not only active in myeloid cells, but also works in the CNS to defend against neurotropic viruses. IL-1β appears to be of particular importance in protecting against WNV infection, because IL-1-deficient mice show a greater viral load in the brain, as compared to peripheral sites such as the spleen, draining lymph nodes, and serum, which have similar viral loads to those of wild type animals [58]. In addition IL-1β has been suggested to synergise with type I IFN to suppress WNV in cortical neurons [58]. Furthermore, mice lacking Nlrp3, caspase 1, or the receptor for IL-1 show reduced survival in response to the virus [58]. In addition, the receptor for IL-1 is required for effective dendritic cell–T cell interactions in response to WNV in the CNS, but not in the periphery [59]. The importance of IL-1β is supported by another report showing that mice lacking ASC are more susceptible to the virus, and analyses of brains from these mice revealed a dysregulated and enhanced inflammatory response [60]. Although most of the data examining immunity to WNV have used mouse models, it has recently been shown that IL-1β is enhanced in the serum of infected patients [58], suggesting an important role for the inflammasome in defence against WNV in both mouse and human systems.

By contrast, for JEV encephalitis there is now evidence for Nlrp3 inflammasome-dependent production of IL-1β and IL-18, which in this context contributes to pathology [61]. Therefore, similar to TLR responses to some viruses in the CNS, activation of the Nlrp3 inflammasome in response to viral infection can either be protective or induce pathology, depending on the type of virus infection.

DNA sensors and STING respond to neurotropic viruses

In recent years it has emerged that DNA is a potent PAMP when localised in the cytoplasm, and several immune sensors of cytosolic DNA have been identified [62]. These include DNA-dependent activator of IFN regulatory factors (DAI), IFI16, DEAD (Asp-Glu-Ala-Asp) box polypeptide 41 (DDX41), cyclic GMP-AMP synthase (cGAS), and DNA-dependent protein kinase [63–67]. In most cases, these sensors require the adaptor protein STING for activation of downstream signalling. STING recruits TBK1, which results in the activation of the transcription factor IRF3 and the production of type I IFNs and proinflammatory cytokines [62]. Several DNA sensors are expressed in the CNS in mice and are further induced after viral CNS entry during infection [27]. In addition, *in vitro* studies have demonstrated expression of STING in astrocytes and microglia cells, and also shown a role for DAI in mediating HSV-1-induced neuronal cell death [68]. STING-deficient mice exhibit elevated viral load in the brain and accelerated death after infection with HSV-1 [69]. A recent study reported the phenotype of mice lacking cGAS; HSV titres in the brain are enhanced and survival is dramatically reduced in these animals [70]. These findings support earlier studies that pointed to cGAS as a central host response to viral DNA, and therefore vital for protection against HSV. However, whether other DNA sensors act in concert or in a cell-type-specific manner with cGAS to induce antiviral immunity is unknown.

Interestingly, it has been reported that HSV-1 specifically targets IFI16 for degradation via the viral E3 ubiquitin ligase infected cell polypeptide 0 (ICP0) [71], suggesting a key role for this sensor in innate control of HSV-1 infection. In addition, accumulating evidence suggests that herpes virus DNA can be sensed by the host in the nucleus during both productive and latent infection to stimulate IFI16-dependent inflammasome activation [72,73]. These

findings raise important questions as to how the host distinguishes between viral and host DNA in the nucleus, and how the host immune responses to nuclear DNA recognition contribute to antiviral defence. In addition, the question of how the host copes with constitutive low-grade inflammasome activation during latency is interesting; in particular given the range of CNS diseases suggested to be associated with latent herpes virus infections.

It is important to note that the function of STING may not be limited to DNA-driven signalling, and in fact STING was demonstrated early on to interact with RIG-I [74], and also to be involved in RIG-I-stimulated signalling under certain conditions of infection with RNA viruses [74]. Moreover, the process of virus–cell fusion triggers innate immune activation in a STING-dependent manner [75]. Such data may explain why STING has been ascribed roles in immune responses to RNA viruses and also why RNA viruses target STING to evade host responses [76,77]. In addition, although DNA is a potent trigger of type I IFN expression, this PAMP also induces other antiviral pathways [62]. Of particular relevance for antiviral defence in the CNS are the findings that cytosolic DNA, such as for example that of HSV-1, activates autophagy via STING, and that autophagy proteins regulate STING function (Box 1) [78].

Collectively, there is now emerging evidence to suggest that the innate DNA sensing pathway is involved in control of viruses in the CNS. However, there is still limited knowledge as to which DNA sensors mediate these responses, which cell types are involved, and what antiviral effector mechanisms exert the DNA-driven antiviral response in the CNS. Finally, it will be interesting to learn how viruses seek to evade the host immune response in the CNS to facilitate establishment and maintenance of infection.

Tissue-specific ISG expression in CNS antiviral responses

The innate antiviral immune response is critically dependent upon the ability of the host to induce effectively the expression of ISGs in response to PRR stimulation, either directly or via type I IFN acting through the IFNAR. Recent studies have highlighted specific roles for individual ISGs in protection against neurotropic viruses. For example it was shown that interferon-induced protein with tetratricopeptide repeats 2 (IFIT2) (also known as ISG54) protects neurons from VSV, yet has no role in conferring protection in the liver and lung, despite the fact that the virus does induce IFIT2 expression in these tissues [79]. This indicates a tissue-specific action of an individual ISG to protect the CNS against viral infection. Why IFIT2 antiviral function is restricted to neurons is unclear, but this finding suggests the existence of a differential ISG-mediated protection in the CNS versus the periphery. Also of note is the report that the CNS displays a region-specific pattern of ISG expression in response to viral infection: granule cell neurons of the cerebellum are more resistant to WNV infection when compared with cortical neurons of mice, likely due to the increased expression of a number of ISGs such as Ifi27, Irg1, Rsad2 (viperin), and Stat1 [80]. Interestingly a similar pattern of protection is observed in the human system, where a fatal WNV infection was shown to infect the cortex to a greater extent compared with the cerebellum [80]. This may suggest a similar differential pattern of ISG expression in the mouse and human system.

Several other ISGs have also been implicated in protective immunity against WNV in the CNS. Two examples are protein kinase (PK)R and RNase L. PKR kinase activity is activated by binding of viral dsRNA. This leads to inhibitory phosphorylation of its substrate, eukaryotic translation initiation factor (eIF)2α, causing protein synthesis to shutdown [81]. In addition, viral dsRNA present in cells also activates 2′-5′-oligoadenylate synthetase (OAS). This enzyme produces 2′-5′ oligoadenylate, which activates the endoribonuclease RNase L. Activation of RNase L results in degradation of both viral RNA and host mRNA, thus inhibiting viral replication and protein synthesis [82]. Mice deficient in both PKR and RNase L showed reduced survival in response to WNV infection and had increased viral burden in the CNS when compared to wild type mice [83]. Both PKR and RNase L were shown to be required for type I IFN to reduce infection in primary macrophages and cortical neurons. However, in peripheral neurons of the superior cervical ganglia, type I IFN did not require PRK and RNase L to control WNV [83]. Indeed, the observation that a missense mutation in 2′-5′ OAS renders mice susceptible to WNV, further supports the notion that the OAS/RNase L pathway defends against WNV [84]. Interestingly, the protein L* from the neurotropic picornavirus Theiler's virus inhibits the IFN-inducible OAS/RNAse L pathway by direct interaction with RNase L [85]. This inhibition was found to be species specific, as L* inhibited mouse RNase L but not orthologs in species including humans [85]. Another ISG that has also been implicated in control of WNV is viperin. Mice lacking viperin show increased lethality to the virus and greater viral replication in the CNS [86]. Viperin displays an expression pattern characteristic of ISGs, and is expressed in both infected and in uninfected neighbouring cells. The mechanism whereby viperin inhibits WNV is unknown, but may involve inhibition of endoplasmic reticulum-dependent protein secretion, regulation of cell survival, or interference with viral protein localisation and viral lipid content [86]. These findings underscore the important antiviral functions of RNase L and viperin in the CNS.

In summary, cells in the CNS have devised a specific system of ISG expression to defend against neurotropic viruses, with a central role for PKR, OAS/RNase L, viperin, and IFIT2. The mechanisms that regulate tissue-specific expression of these pathways will be an important area of future investigation.

Concluding remarks

In recent years significant progress has been made in understanding innate antiviral signalling mechanisms in the CNS. It is clear that many of the mechanisms that have been characterised in the periphery are also present and functional in the CNS. Much of what we now know about these pathways has been obtained from work in mice. Therefore, there is a clear need to increase our knowledge of the human system, using either cultured human neurons or more studies of individuals susceptible to neurotropic viruses.

Several important questions remain to be addressed. In response to at least two viruses, VSV and WNV, mice lacking MyD88 demonstrated a more significant role for

this key adaptor in providing immunity to a greater extent than that of TLR7; the TLR that would be expected to sense these viruses upstream of MyD88. This suggest that a TLR-independent role for MyD88, possibly through IL-1β or IL-18 signalling, is important for antiviral responses in the context of these infections. Alternatively, MyD88 may act downstream of proposed DExD/H box helicases nucleic acid sensors in the CNS [87,88]. In addition, although it is already known that microglia–neuron interactions are important in situations of health and disease [89], the cellular communication events important in responding to viral infection in the CNS remain to be clarified. For example SARM-dependent responses to VSV require interactions between microglia and neurons, the exact nature of which are unclear [41].

Although many PRRs contribute to antiviral responses, two classes of PRRs are capable of driving both neuroprotection and neuropathology in response to CNS viruses – TLRs and the Nlrp3 inflammasome. This article discusses the theme of 'appropriate' immunity during viral infection. For example, although MAVS and ASC are both important for immunity against WNV, they are also required to prevent an exaggerated immune response, indicative of a regulatory role. It will be important to uncover the precise mechanisms underlying these processes. In the CNS, postmitotic cells such as neurons are more likely to use autophagy as a means of antiviral defence rather than cytolytic mechanisms [56]. Therefore methods that enhance autophagy but limit proinflammatory mediators are likely to be of benefit during viral CNS infections. Future work should reveal the molecular and cellular events that underlie viral CNS infections, and in doing so reveal novel therapeutic opportunities.

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References

1 Janeway, C.A., Jr (1989) Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb. Symp. Quant. Biol.* 54, 1–13
2 Palm, N.W. and Medzhitov, R. (2009) Pattern recognition receptors and control of adaptive immunity. *Immunol. Rev.* 227, 221–233
3 de Veer, M.J. *et al.* (2001) Functional classification of interferon-stimulated genes identified using microarrays. *J. Leukoc. Biol.* 69, 912–920
4 Menager, P. *et al.* (2009) Toll-like receptor 3 (TLR3) plays a major role in the formation of rabies virus Negri bodies. *PLoS Pathog.* 5, e1000315
5 Takeuchi, O. and Akira, S. (2010) Pattern recognition receptors and inflammation. *Cell* 140, 805–820
6 Peltier, D.C. *et al.* (2010) Human neuronal cells possess functional cytoplasmic and TLR-mediated innate immune pathways influenced by phosphatidylinositol-3 kinase signaling. *J. Immunol.* 184, 7010–7021
7 O'Neill, L.A. *et al.* (2013) The history of Toll-like receptors - redefining innate immunity. *Nat. Rev. Immunol.* 13, 453–460
8 Kawai, T. and Akira, S. (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 34, 637–650
9 O'Neill, L.A. and Bowie, A.G. (2007) The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat. Rev. Immunol.* 7, 353–364
10 Carty, M. and Bowie, A.G. (2011) Evaluating the role of Toll-like receptors in diseases of the central nervous system. *Biochem. Pharmacol.* 81, 825–837
11 Oshiumi, H. *et al.* (2011) The TLR3/TICAM-1 pathway is mandatory for innate immune responses to poliovirus infection. *J. Immunol.* 187, 5320–5327
12 Shi, Z. *et al.* (2011) A novel Toll-like receptor that recognizes vesicular stomatitis virus. *J. Biol. Chem.* 286, 4517–4524
13 Lang, K.S. *et al.* (2007) MyD88 protects from lethal encephalitis during infection with vesicular stomatitis virus. *Eur. J. Immunol.* 37, 2434–2440
14 Daffis, S. *et al.* (2008) Toll-like receptor 3 has a protective role against West Nile virus infection. *J. Virol.* 82, 10349–10358
15 Wang, T. *et al.* (2004) Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nat. Med.* 10, 1366–1373
16 Town, T. *et al.* (2009) Toll-like receptor 7 mitigates lethal West Nile encephalitis via interleukin 23-dependent immune cell infiltration and homing. *Immunity* 30, 242–253
17 Welte, T. *et al.* (2009) Toll-like receptor 7-induced immune response to cutaneous West Nile virus infection. *J. Gen. Virol.* 90, 2660–2668
18 Szretter, K.J. *et al.* (2010) The innate immune adaptor molecule MyD88 restricts West Nile virus replication and spread in neurons of the central nervous system. *J. Virol.* 84, 12125–12138
19 Xie, G. *et al.* (2013) A West Nile virus NS4B-P38G mutant strain induces adaptive immunity via TLR7-MyD88-dependent and independent signaling pathways. *Vaccine* 31, 4143–4151
20 Paludan, S.R. *et al.* (2011) Recognition of herpesviruses by the innate immune system. *Nat. Rev. Immunol.* 11, 143–154
21 Zhang, S.Y. *et al.* (2007) TLR3 deficiency in patients with herpes simplex encephalitis. *Science* 317, 1522–1527
22 Sancho-Shimizu, V. *et al.* (2011) Herpes simplex encephalitis in children with autosomal recessive and dominant TRIF deficiency. *J. Clin. Invest.* 121, 4889–4902
23 Perez de Diego, R. *et al.* (2010) Human TRAF3 adaptor molecule deficiency leads to impaired Toll-like receptor 3 response and susceptibility to herpes simplex encephalitis. *Immunity* 33, 400–411
24 Herman, M. *et al.* (2012) Heterozygous TBK1 mutations impair TLR3 immunity and underlie herpes simplex encephalitis of childhood. *J. Exp. Med.* 209, 1567–1582
25 Casrouge, A. *et al.* (2006) Herpes simplex virus encephalitis in human UNC-93B deficiency. *Science* 314, 308–312
26 Guo, Y. *et al.* (2011) Herpes simplex virus encephalitis in a patient with complete TLR3 deficiency: TLR3 is otherwise redundant in protective immunity. *J. Exp. Med.* 208, 2083–2098
27 Reinert, L.S. *et al.* (2012) TLR3 deficiency renders astrocytes permissive to herpes simplex virus infection and facilitates establishment of CNS infection in mice. *J. Clin. Invest.* 122, 1368–1376
28 Menasria, R. *et al.* (2013) Both TRIF and IPS-1 adaptor proteins contribute to the cerebral innate immune response against herpes simplex virus 1 infection. *J. Virol.* 87, 7301–7308
29 Lafaille, F.G. *et al.* (2012) Impaired intrinsic immunity to HSV-1 in human iPSC-derived TLR3-deficient CNS cells. *Nature* 491, 769–773
30 Weber, F. *et al.* (2006) Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. *J. Virol.* 80, 5059–5064
31 Schulz, O. *et al.* (2005) Toll-like receptor 3 promotes cross-priming to virus-infected cells. *Nature* 433, 887–892
32 Xu, Y. *et al.* (2007) Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. *Immunity* 27, 135–144
33 Orvedahl, A. *et al.* (2007) HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. *Cell Host Microbe* 1, 23–35
34 Johnson, N. *et al.* (2010) The immune response to rabies virus infection and vaccination. *Vaccine* 28, 3896–3901
35 Chopy, D. *et al.* (2011) Ambivalent role of the innate immune response in rabies virus pathogenesis. *J. Virol.* 85, 6657–6668
36 Liberati, N.T. *et al.* (2004) Requirement for a conserved Toll/interleukin-1 resistance domain protein in the *Caenorhabditis elegans* immune response. *Proc. Natl. Acad. Sci. U.S.A.* 101, 6593–6598
37 Chang, C. *et al.* (2011) Microtubule-based localization of a synaptic calcium-signaling complex is required for left-right neuronal asymmetry in *C. elegans*. *Development* 138, 3509–3518
38 Couillault, C. *et al.* (2004) TLR-independent control of innate immunity in *Caenorhabditis elegans* by the TIR domain adaptor protein TIR-1, an ortholog of human SARM. *Nat. Immunol.* 5, 488–494
39 Carty, M. *et al.* (2006) The human adaptor SARM negatively regulates adaptor protein TRIF-dependent Toll-like receptor signaling. *Nat. Immunol.* 7, 1074–1081
40 Kim, Y. *et al.* (2007) MyD88-5 links mitochondria, microtubules, and JNK3 in neurons and regulates neuronal survival. *J. Exp. Med.* 204, 2063–2074
41 Hou, Y.J. *et al.* (2013) SARM is required for neuronal injury and cytokine production in response to central nervous system viral infection. *J. Immunol.* 191, 875–883

42 Mukherjee, P. *et al.* (2013) Activation of the innate signaling molecule MAVS by bunyavirus infection upregulates the adaptor protein SARM1, leading to neuronal death. *Immunity* 38, 705–716
43 Szretter, K.J. *et al.* (2009) The immune adaptor molecule SARM modulates tumor necrosis factor alpha production and microglia activation in the brainstem and restricts West Nile Virus pathogenesis. *J. Virol.* 83, 9329–9338
44 Osterloh, J.M. *et al.* (2012) dSarm/Sarm1 is required for activation of an injury-induced axon death pathway. *Science* 337, 481–484
45 Gerdts, J. *et al.* (2013) Sarm1-mediated axon degeneration requires both SAM and TIR interactions. *J. Neurosci.* 33, 13569–13580
46 Schlee, M. (2013) Master sensors of pathogenic RNA - RIG-I like receptors. *Immunobiology* 218, 1322–1335
47 Gurtler, C. and Bowie, A.G. (2013) Innate immune detection of microbial nucleic acids. *Trends Microbiol.* 21, 413–420
48 Faul, E.J. *et al.* (2010) Rabies virus infection induces type I interferon production in an IPS-1 dependent manner while dendritic cell activation relies on IFNAR signaling. *PLoS Pathog.* 6, e1001016
49 Rothenfusser, S. *et al.* (2005) The RNA helicase Lgp2 inhibits TLR-independent sensing of viral replication by retinoic acid-inducible gene-I. *J. Immunol.* 175, 5260–5268
50 Satoh, T. *et al.* (2010) LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1512–1517
51 Lafon, M. *et al.* (2008) Detrimental contribution of the immuno-inhibitor B7-H1 to rabies virus encephalitis. *J. Immunol.* 180, 7506–7515
52 Suthar, M.S. *et al.* (2010) IPS-1 is essential for the control of West Nile virus infection and immunity. *PLoS Pathog.* 6, e1000757
53 Fredericksen, B.L. *et al.* (2008) Establishment and maintenance of the innate antiviral response to West Nile Virus involves both RIG-I and MDA5 signaling through IPS-1. *J. Virol.* 82, 609–616
54 Wollish, A.C. *et al.* (2013) An attenuating mutation in a neurovirulent Sindbis virus strain interacts with the IPS-1 signaling pathway in vivo. *Virology* 435, 269–280
55 Nazmi, A. *et al.* (2011) RIG-I mediates innate immune response in mouse neurons following Japanese encephalitis virus infection. *PLoS ONE* 6, e21761
56 Orvedahl, A. *et al.* (2010) Autophagy protects against Sindbis virus infection of the central nervous system. *Cell Host Microbe* 7, 115–127
57 Allen, I.C. *et al.* (2009) The NLRP3 inflammasome mediates in vivo innate immunity to influenza A virus through recognition of viral RNA. *Immunity* 30, 556–565
58 Ramos, H.J. *et al.* (2012) IL-1beta signaling promotes CNS-intrinsic immune control of West Nile virus infection. *PLoS Pathog.* 8, e1003039
59 Durrant, D.M. *et al.* (2013) IL-1R1 is required for dendritic cell-mediated T cell reactivation within the CNS during West Nile virus encephalitis. *J. Exp. Med.* 210, 503–516
60 Kumar, M. *et al.* (2013) Inflammasome adaptor protein apoptosis-associated speck-like protein containing CARD (ASC) is critical for the immune response and survival in west Nile virus encephalitis. *J. Virol.* 87, 3655–3667
61 Kaushik, D.K. *et al.* (2012) NLRP3 inflammasome: key mediator of neuroinflammation in murine Japanese encephalitis. *PLoS ONE* 7, e32270
62 Paludan, S.R. and Bowie, A.G. (2013) Immune sensing of DNA. *Immunity* 38, 870–880
63 Takaoka, A. *et al.* (2007) DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature* 448, 501–505
64 Unterholzner, L. *et al.* (2010) IFI16 is an innate immune sensor for intracellular DNA. *Nat. Immunol.* 11, 997–1004
65 Zhang, Z. *et al.* (2011) The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat. Immunol.* 12, 959–965
66 Sun, L. *et al.* (2013) Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science* 339, 786–791
67 Ferguson, B.J. *et al.* (2012) DNA-PK is a DNA sensor for IRF-3-dependent innate immunity. *ELife* 1, e00047
68 Furr, S.R. *et al.* (2011) A role for DNA-dependent activator of interferon regulatory factor in the recognition of herpes simplex virus type 1 by glial cells. *J. Neuroinflammation* 8, 99
69 Ishikawa, H. *et al.* (2009) STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature* 461, 788–792
70 Li, X.D. *et al.* (2013) Pivotal roles of cGAS-cGAMP signaling in antiviral defense and immune adjuvant effects. *Science* 341, 1390–1394
71 Orzalli, M.H. *et al.* (2012) Nuclear IFI16 induction of IRF-3 signaling during herpesviral infection and degradation of IFI16 by the viral ICP0 protein. *Proc. Natl. Acad. Sci. U.S.A.* 109, E3008–E3017
72 Ansari, M.A. *et al.* (2013) Constitutive interferon-inducible protein 16-inflammasome activation during Epstein-Barr virus latency I, II, and III in B and epithelial cells. *J. Virol.* 87, 8606–8623
73 Johnson, K.E. *et al.* (2013) Herpes simplex virus 1 infection induces activation and subsequent inhibition of the IFI16 and NLRP3 inflammasomes. *J. Virol.* 87, 5005–5018
74 Ishikawa, H. and Barber, G.N. (2008) STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature* 455, 674–678
75 Holm, C.K. *et al.* (2012) Virus-cell fusion as a trigger of innate immunity dependent on the adaptor STING. *Nat. Immunol.* 13, 737–743
76 Aguirre, S. *et al.* (2012) DENV inhibits type I IFN production in infected cells by cleaving human STING. *PLoS Pathog.* 8, e1002934
77 Nitta, S. *et al.* (2013) Hepatitis C virus NS4B protein targets STING and abrogates RIG-I-mediated type I interferon-dependent innate immunity. *Hepatology* 57, 46–58
78 Rasmussen, S.B. *et al.* (2011) Activation of autophagy by alpha-herpesviruses in myeloid cells is mediated by cytoplasmic viral DNA through a mechanism dependent on stimulator of IFN genes. *J. Immunol.* 187, 5268–5276
79 Fensterl, V. *et al.* (2012) Interferon-induced Ifit2/ISG54 protects mice from lethal VSV neuropathogenesis. *PLoS Pathog.* 8, e1002712
80 Cho, H. *et al.* (2013) Differential innate immune response programs in neuronal subtypes determine susceptibility to infection in the brain by positive-stranded RNA viruses. *Nat. Med.* 19, 458–464
81 Meurs, E.F. *et al.* (1992) Constitutive expression of human double-stranded RNA-activated p68 kinase in murine cells mediates phosphorylation of eukaryotic initiation factor 2 and partial resistance to encephalomyocarditis virus growth. *J. Virol.* 66, 5805–5814
82 Silverman, R.H. (2007) Viral encounters with 2′,5′-oligoadenylate synthetase and RNase L during the interferon antiviral response. *J. Virol.* 81, 12720–12729
83 Samuel, M.A. *et al.* (2006) PKR and RNase L contribute to protection against lethal West Nile Virus infection by controlling early viral spread in the periphery and replication in neurons. *J. Virol.* 80, 7009–7019
84 Mashimo, T. *et al.* (2002) A nonsense mutation in the gene encoding 2′-5′-oligoadenylate synthetase/L1 isoform is associated with West Nile virus susceptibility in laboratory mice. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11311–11316
85 Sorgeloos, F. *et al.* (2013) Evasion of antiviral innate immunity by Theiler's virus L* protein through direct inhibition of RNase L. *PLoS Pathog.* 9, e1003474
86 Szretter, K.J. *et al.* (2011) The interferon-inducible gene viperin restricts West Nile virus pathogenesis. *J. Virol.* 85, 11557–11566
87 Kim, T. *et al.* (2010) Aspartate-glutamate-alanine-histidine box motif (DEAH)/RNA helicase A helicases sense microbial DNA in human plasmacytoid dendritic cells. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15181–15186
88 Zhang, Z. *et al.* (2011) DDX1, DDX21, and DHX36 helicases form a complex with the adaptor molecule TRIF to sense dsRNA in dendritic cells. *Immunity* 34, 866–878
89 Biber, K. *et al.* (2008) Neuron-microglia signaling: chemokines as versatile messengers. *J. Neuroimmunol.* 198, 69–74
90 Yordy, B. *et al.* (2012) A neuron-specific role for autophagy in antiviral defense against herpes simplex virus. *Cell Host Microbe* 12, 334–345
91 Kurt-Jones, E.A. *et al.* (2004) Herpes simplex virus 1 interaction with Toll-like receptor 2 contributes to lethal encephalitis. *Proc. Natl. Acad. Sci. U.S.A.* 101, 1315–1320
92 Lazear, H.M. *et al.* (2013) The pattern recognition receptor MDA5 modulates CD8+ T cell-dependent clearance of West Nile virus from the central nervous system. *J. Virol.* 87, 11401–11415
93 Choi, A.M. *et al.* (2013) Autophagy in human health and disease. *N. Engl. J. Med.* 368, 651–662
94 Saitoh, T. *et al.* (2009) Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. *Proc. Natl. Acad. Sci. U.S.A.* 106, 20842–20846