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·Review·

Innate immune responses regulate morphogenesis and degeneration: roles of Toll-like receptors and Sarm1 in neurons

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The central nervous system is recognized as an immunoprivileged site because peripheral immune cells do not typically enter it. Microglial cells are thought to be the main immune cells in brain. However, recent reports have indicated that neurons express the key players of innate immunity, including Toll-like receptors (TLRs) and their adaptor proteins (Sarm1, Myd88, and Trif), and may produce cytokines in response to pathogen infection. In the absence of an immune challenge, neuronal TLRs can detect intrinsic danger signals and modulate neuronal morphology and function. In this article, we review the recent findings on the involvement of TLRs and Sarm1 in controlling neuronal morphogenesis and neurodegeneration. Abnormal behaviors in TLR- and Sarm1-deficient mice are also discussed.

Keywords: axon; cytokines; dendrite; innate immunity; interleukin-6; Sarm1; toll-like receptor

Introduction

The innate immune system recognizes pathogenic molecules derived from bacteria and viruses and activates the expression of various antiviral and inflammatory cytokines, the complement cascade, and phagocytosis to eliminate foreign pathogens. Distinct from adaptive immunity, innate immunity lacks antigen specificity. It uses pattern recognition receptors to identify pathogen-associated molecular patterns, including lipopolysaccharide, lipopeptides, flagellin, and single- and double-stranded RNA and DNA[1]. In addition to foreign molecules, these pattern recognition receptors can also recognize endogenous ligands, which are released from cells and tissues undergoing stress or injury[2]. This results in either chronic or acute inflammatory responses in the absence of pathogen infection. Thus, innate immunity serves as an alarm system that responds to both exogenous pathogens and endogenous damage signals.

Toll-like receptors (TLRs), the most well-studied pattern-recognition receptors, play critical roles in the initiation of innate immune responses. At least 13 TLRs have been identified in mammals. Different TLRs recognize distinct molecular patterns. Based on their subcellular localization, TLRs can be separated into two categories. The first group, containing TLR1, TLR2, TLR4, TLR5, and TLR6, is expressed on the cell surface. The second category, containing TLR3, TLR7, TLR8, TLR9, and TLR13, is localized to the intracellular endosomal compartment[3].

TLRs are widely expressed in various types of cells. Microglia, the specialized immune cells in the brain, constitutively express a broad array of TLRs[4, 5]. The most well-studied TLRs in microglia are TLR2 and TLR4 that are key players in neuroinflammation in CNS trauma and neurodegenerative disease[6]. TLR2 and TLR4 signaling induces microglia activation after brain injury or pathogen infection, and this produces various pro-inflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor α (TNFα), type I interferon (IFN), and IL-1β. The exacerbated inflammation in brain causes neuronal loss and brain damage[7]. In the past decade, the accumulated evidence

suggests that neurons do have innate immunity. The importance and biological meaning of the neuronal innate immune responses have recently been investigated. In this review, we focus on the function of neuronal TLRs and their downstream effectors in neuronal development and neurodegeneration.

TLRs and Toll/interleukin-1 Receptor (TIR) Domain-containing Adaptors in Neurons

TLR expression and activation have been reported in both neuronal cell lines and primary cultured neurons. The human NT2-N neuronal cell line expresses TLR1, TLR2, TLR3, and TLR4[8]. Activation of TLR3 using poly I:C, a synthetic double-stranded RNA (dsRNA), induces the expression of antiviral and inflammatory cytokines, including IFN-β, CCL-5, CCL-10, TNFα, and IL-6 in NT2-N cells[8]. Similarly, rodent neurons express a variety of TLRs and their downstream effectors. Activation of TLR4 using lipopolysaccharide (LPS) induces CCL5, CXCL1, TNFα, and IL-6 production in mouse cortical neurons[9]. Furthermore, Kaul and colleagues performed quantitative PCR to examine the expression levels of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR9 in developing brains and found that the levels of TLR7 and TLR9 correlated particularly well with brain development[10]. Our recent study also indicated that activation of neuronal TLR7 induces both mRNA and protein expression of IL-6 and TNFα[11]. In addition to TLRs, neurons also express the critical TIR domain-containing adaptors, which transduce the downstream signals of TLRs, including myeloid differentiation primary response gene 88 (MyD88), TIR domain-containing adapter-inducing IFN-β (Trif)[10] and sterile alpha and HEAT/Armadillo motif-containing 1 (Sarm1)[12]. Notably, Sarm1 is predominantly expressed in neurons rather than astrocytes, microglia, or the peripheral immune system[12-14]. This characteristic is unique to Sarm1 among all of the known TLRs and TIR domain-containing adaptors. Indeed, Sarm1 plays multiple roles in the nervous system, and this will be discussed in following sections. Based on these lines of evidence, it is clear that neurons express various TLRs and TIR domain-containing adaptors and that activation of neuronal TLRs regulates the expression of various cytokines.

Endosomal TLRs and Their Ligands

In addition to sensing foreign pathogens, TLRs respond to intrinsic damage signals[15]. The exogenous and endogenous ligands specific to the various TLRs have been summarized in several reviews[3, 16]. Here, we are particularly interested in the endosomal TLRs (TLR3, TLR7, TLR8, and TLR9) because they recognize nucleic acids. TLR3 recognizes dsRNA, TLR7 and TLR8 are activated by single-stranded RNA (ssRNA), and TLR9 binds to unmethylated CpG DNA. All four are localized to the endosomal pathway, by which they interact with their ligands. Foreign bacteria and viruses are internalized and digested *via* the endosomal pathway. The bacterial and viral nucleic acids then interact with the endosomal TLRs in the intracellular vesicular compartments and activate innate immune responses, including the expression of anti-viral and inflammatory cytokines. Several studies suggest that self nucleic acids can be ligands of TLR3, TLR7, TLR8, and TLR9[2]. For instance, heterologous RNAs released from necrotic cells or generated by *in vitro* transcription activate the TLR3 signaling pathway and induce IL-8 secretion[17]. *In vivo*, TLR3 is required for injury-induced acute inflammatory responses. During experimental polymicrobial septic peritonitis and ischemic gut injury, the levels of inflammatory cytokines quickly drop to baseline in TLR3-deficient mice[18]. Thus, dying cells (both apoptotic and necrotic) are sources that provide self nucleic acids to activate endosomal TLRs.

Some reports have specified the types of endogenous ligands for TLR3 and TLR7 binding. RNAs containing a high degree of self-complementarity target TLR3, whereas TLR7 is activated by uridine-rich RNAs[19]. Moreover, TLR7 recognizes microRNAs (miRNAs), particularly let-7[20], miR-21, and miR-29a[21]. Because microRNAs are present in exosomes[22,23], it has been suggested that cells release miRNA into the environment *via* exosomes, activating TLR7 in other cells[20, 21]. Interestingly, in this model, the effect of miRNA on other cells is not *via* the canonical pathway, in which miRNAs complementarily bind to mRNA and reduce the expression of the targeted mRNA. Instead, the internalized exosomal miRNAs enter the endosomal pathway, are consequently released from exosomes, and activate TLR7 in the intracellular vesicular compartments (Fig. 1). Through this mechanism, TLR7 may receive

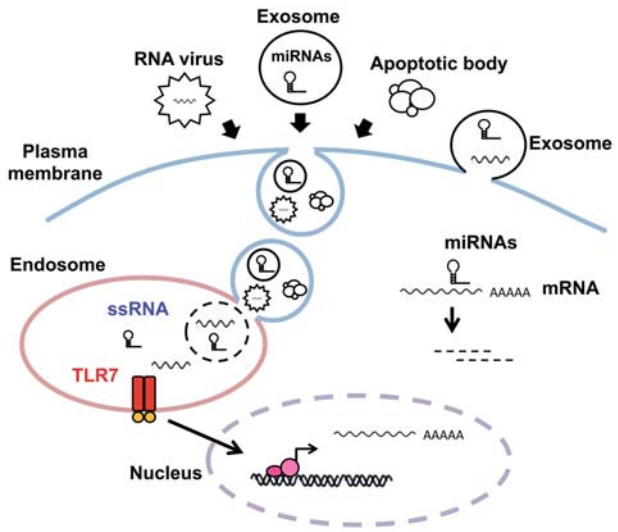

Fig. 1. Exogenous and endogenous ligands of TLR7. Both exogenous and endogenous ligands are recognized by TLR7 *via* the endosomal pathway. Endogenous ligands can be delivered *via* apoptotic bodies or exosomes. Exosomes containing proteins, mRNAs, and miRNAs are released from cells and travel either a short or long distance to influence the activity of target cells. All viruses, bacteria, apoptotic bodies, and exosomes can be internalized and enter the endosomal pathway. In endosomes, partially-digested ssRNAs, including mRNAs and miRNAs, are recognized by TLR7, thus triggering innate immune responses. In this model, miRNAs perform a novel function, the activation of the TLR7 signaling pathway, rather than directly silencing gene expression.

signals from distant cells and trigger an innate immune response.

The known pathogenic, synthetic, and endogenous ligands for TLR3, TLR7, TLR8, and TLR9 are summarized in Table 1. It should be noted that although imiquimod (termed R837, an imidazoquinoline compound), CL075 (a thiazoquinoline compound) and loxoribine (a guanosine analog) have all been commonly used as TLR7 agonists[24-27], several studies have indicated non-specific effects of imiquimod on neurons. We have shown that imiquimod, CL075, and loxoribine restrict dendrite growth in wild-type rodent cortical and hippocampal neurons[11]. However, CL075 and loxoribine lose their effects in TLR7-knockout neurons, suggesting an essential role of TLR7 in the efficacy of CL075 and loxoribine with respect to neuronal morphology. In contrast, the ability of imiquimod to restrict dendrite growth is not affected by TLR7-knockout[11]. This result suggests that TLR7 is not the only target of imiquimod in cortical and hippocampal neurons. In dorsal root ganglion neurons, it has been shown that imiquimod treatment results in the activation of transient receptor potential vanilloid 1 and the inhibition of background and voltage-gated K^+ channels, which are TLR7-independent[28,29]. These independent results indicate that the specificity of imiquimod for TLR7 is a concern, at least regarding neurons. Our study suggested that CL075 and loxoribine are more specific to TLR7[11]. Thus, to study the function of TLR7 in neurons, imiquimod (R837) should be avoided to minimize non-specific effects.

TLRs and Neuronal Morphogenesis

In neurons, the activation of TLR pathways likely performs multiple functions. Similar to other types of cells, the activation of TLRs in neurons induces the expression of cytokines, as described above. Moreover, evidence indicates that TLR activation is also critical for neuronal morphogenesis. In cultured dorsal root ganglion, cortical, and hippocampal neurons, treatment with poly I:C, a synthetic dsRNA, induces growth-cone collapse and inhibits neurite outgrowth[30]. The effect of poly I:C is mediated *via* TLR3 because neurons lacking functional TLR3 do

Table 1. Summary of the pathogenic, synthetic, and endogenous ligands of mouse TLR3, TLR7, TLR8, and TLR9

	Pathogenic ligands	Synthetic ligands	Endogenous ligands
TLR3	dsRNA	Poly IC	mRNA[30, 61]
TLR7	ssRNA	CL075, Loxoribine, R848[11, 20, 21, 32]	let-7, miR-21, miR-29a[20, 21, 32]
TLR8		R848[31]	?
TLR9	Unmethylated CpG DNA	CpG ODN	DNA

not respond to poly I:C[30]. This study indicates that TLR3 activation plays a negative role in neurite outgrowth.

In addition to TLR3, TLR7 and TLR8 have also been suggested to negatively regulate neurite outgrowth in mouse cortical neurons[11, 31]. Ma and colleagues showed that R848, an imidazoquinoline compound, inhibits neurite outgrowth and triggers apoptosis in cortical neurons[31]. Because R848 can activate TLR7 as well as TLR8, Ma and colleagues then used antibodies to examine the expression of TLR7 and TLR8 in cortical neurons, and their data suggested that only TLR8, and not TLR7, is expressed. Thus, they concluded that the negative effect of R848 is mediated *via* TLR8 but not TLR7[31]. However, their results are in conflict to many recent studies from various laboratories regarding the expression of TLR7 in neurons[10, 11, 20, 32], as evidenced by the results of *in situ* hybridization, quantitative polymerase chain reaction (Q-PCR), and immunostaining using TLR7 antibodies[10, 11, 20, 32]. Actually, the expression level of TLR7 in neurons is even higher than that of TLR8[11]. Therefore, the R848 treatment in Ma's study likely activates both TLR7 and TLR8 in neurons to inhibit neurite growth. To determine the role of TLR8 in neuronal morphogenesis, additional investigations using specific agonists and genetic manipulations (such as knockout mice and RNAi-mediated knockdown) are needed. As for the role of TLR7 in neuronal morphogenesis, we have demonstrated that TLR7-knockout neurons have longer axons and dendrites, and reintroduction of TLR7 into TLR7-deficient neurons rescues this overextension[11]. Moreover, activation of TLR7 by TLR7-specific agonists (CL075 and loxoribine) negatively regulates dendritic growth in wild-type but not in TLR7-deficient neurons. In Ma's study, they applied 500 μmol/L loxoribine to cultured neurons and did not detect a negative effect on neurite outgrowth[31]. We used 1 mmol/L loxoribine and did find a reduction of dendritic length[11]. Possibly, loxoribine is relatively inefficient, and a higher concentration may be required to activate TLR7. Both genetic manipulation and pharmaceutical treatment support a function of TLR7 in neuronal morphogenesis.

TLR Downstream Signaling in Regulation of Neuronal Morphology

To trigger innate immune responses, two key TIR domain-containing adaptors, MyD88 and Trif, are involved in the canonical TLR pathways. TLR3 transduces signals *via* Trif, TLR4 uses both Myd88 and Trif to activate downstream signals, and the remaining TLRs use MyD88 as their adaptor. The signals may go through NF-κB, interferon regulatory factors (IRFs), and the AP-1 family to induce the expression of inflammatory cytokines and interferons. The detailed signaling pathways of TLRs are available in previous reviews[3, 16, 33, 34].

With respect to the negative regulation of neuronal morphogenesis, only TLR7 signaling is well understood. TLR7 uses a canonical pathway, namely the MyD88-dependent pathway, to induce IL-6 expression in cultured cortical and hippocampal neurons[11]. MyD88 is essential for the capacity of TLR7 to regulate dendrite growth, as MyD88 knockout neurons do not respond to CL075 stimulation. Interestingly, in addition to IL-6 activation, TLR7 activation in neurons also induces the mRNA expression of TNFα and IL-1β, but not IFNβ. At the protein level, only IL-6 and TNFα are detectable in the culture supernatant[11]. Because IL-1β requires a second signal to activate the inflammasome and caspase 1 to cleave pro-IL-1β to IL-1β[35, 36], the undetectable level of IL-1β in the supernatant is likely due to the lack of a second stimulus to activate the inflammasome. Between IL-6 and TNFα, only IL-6 is critical for restricting the dendritic outgrowth of cultured neurons because IL-6 knockout neurons lose their response to TLR7 activation[11]. In contrast, TNFα-knockout neurons remain sensitive to TLR7 activation of dendrite outgrowth[11]. This result suggests that TNFα is not required for the effect of TLR7 on neuronal morphology (Fig. 2). Notably, a previous study indicated that adding exogenous IL-6 or TNFα is sufficient to inhibit dendrite development in cultured cortical neurons[37]. It is not clear what is responsible for this contrasting result. One possibility is the doses used. In the exogenous experiment, 100 U TNFα was added, which is ~2000 pg/mL[37]. When cultured neurons were treated with CL075 to activate TLR7, only 6–8 pg/mL of TNFα was detected in the supernatant[11], which is ~0.4% of the concentration applied exogenously. Interestingly, the concentration of IL-6 in the supernatant of TLR7-activated neurons was even lower (0.6–0.9 pg/mL), which is ~0.001% that of the exogenously-applied IL-6 (~80 000 pg/mL)[11, 37]. It is unclear whether IL-6 synthesized *de novo* in cultured neurons results in more potent activation of the IL-6 receptor. Additional investigations are needed to address this issue.

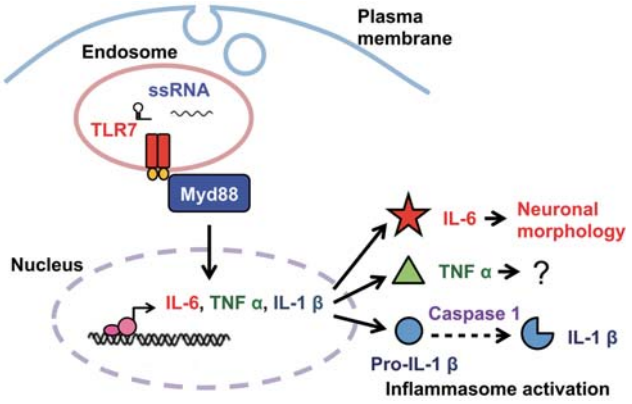

Fig. 2. The signaling pathway downstream of TLR7 in neurons. In cultured cortical and hippocampal neurons, activation of TLR7 induces IL-6, TNFα and pro-IL-1β expression *via* a MyD88-dependent mechanism. Due to the lack of a secondary signal, pro-IL-1β cannot be processed to IL-1β. Both IL-6 and TNFα are released into the culture medium. However, only IL-6 negatively regulates dendrite outgrowth. The role of TNFα in this process is unclear.

In contrast to TLR7, the signaling pathway downstream of TLR3 that controls neurite outgrowth remains to be elucidated[30]. Poly I:C treatment inhibits neurite outgrowth in a TLR3-dependent but NF-κB-independent manner[30]. In this study, MyD88 knockout neurons were also used to demonstrate that MyD88 is not involved in the TLR3 pathway[30]. However, because TLR3 delivers its signals using Trif but not MyD88, it appears to be more appropriate to examine the role of Trif rather than MyD88 in the TLR3 pathway to control neurite outgrowth. It is also unclear whether cytokines are involved downstream of TLR3 in neurons.

Role of TLR7 in Neurodegeneration

In addition to morphogenesis, the expression of TLR may also play a role in neurodegeneration. The studies contributed by Dr. Seija Lehnardt's laboratory unexpectedly revealed that TLR7 recognizes the miRNA let-7, consequently resulting in neurodegeneration[20, 32]. They reported that let-7 released from dying cells activates TLR7 expression in neurons, triggering neuronal death *in vitro* and *in vivo*[20, 32]. Similar to the findings regarding neuronal morphogenesis, MyD88 is required for the function of TLR7 in neurodegeneration, as MyD88 knockout neurons do not respond to let-7[20]. However, it is not clear whether cytokines, such as IL-6 and TNFα, are involved. Interestingly, microglia are not involved in the effect of let-7 on neurodegeneration, as depletion of microglia *via* the expression of thymidine kinase of *Herpes simplex* virus under the control of the CD11 promoter does not influence the effect of let-7 on neuronal death[20]. This result suggests that neuronal TLR7 plays a predominant role in the response to let-7. Related to neurodegenerative disease, they further found that patients with Alzheimer's disease exhibit a higher copy number of let-7 in the cerebrospinal fluid[20]. This was the first study to demonstrate that the recognition of self miRNA by neuronal TLR7 is critical for triggering neurodegeneration.

Innate Immune Responses of CNS Cells: Neurons *versus* Glia

As described above, both neurons and microglia express various TLRs and produce cytokines after their activation. However, the efficiency of cytokine production is much reduced in neurons as compared with microglial cells. Activation of TLRs in neurons produces a very low level of pro-inflammatory cytokines[11, 38, 39], while microglia and astrocytes secret large amounts of cytokines[40, 41]. The low level of cytokine production by neurons seems unlikely to induce a global innate immune response in the brain. Therefore, this raises the question of why neurons need their own innate immunity. Based on current knowledge, we propose that TLRs in neurons function as chemorepulsive sensors. During development, programmed cell death occurs frequently but locally while neurons extend their axons and dendrites[42, 43]. Activation of neuronal TLRs by RNA and/or DNA derived from dead cells may prevent axon and dendrite growth into an unhealthy area through a cell-autonomous mechanism or paracrine signaling[11, 30]. The cytokines produced by neurons may be just enough to recruit and activate local microglia, which in turn engulf the debris of dead cells and do not cause global inflammation. Axons and dendrites then may grow into or pass through the cleaned-up area and establish proper neuronal circuits with healthy neurons. Therefore, the biological meaning and function of TLR activation in neurons could be distinct from that in microglia during development.

TLRs and Mouse Behaviors

Several studies using mouse genetic models have explored the roles of TLRs in learning/memory and sensory and motor behaviors. The first example is TLR3-knockout mice[44]. In the Morris water maze, novel object recognition, and contextual fear conditioning, TLR3-deficient mice exhibit enhanced hippocampus-dependent memory. Interestingly, amygdala-dependent learning and memory are impaired in these mice. Anxiety-related behaviors, which are strongly associated with the amygdala, are also reduced in TLR3-deficient mice[44]. It is not clear why TLR3 deficiency enhances hippocampus-dependent performance but impairs amygdala-dependent behaviors. Additional studies are needed to elucidate the roles of TLR3 in different brain regions.

The behaviors of TLR4-knockout mice have also been analyzed [45]. In the Morris water maze, these mice travel a much shorter distance to locate the hidden platform, suggesting that deletion of TLR4 enhances the acquisition of hippocampus-dependent spatial learning and memory. However, these mice exhibit a lower frequency of the freezing response in contextual fear conditioning, suggesting an impairment of contextual fear conditioning, another hippocampus-dependent spatial learning paradigm. It is unclear why TLR4 deletion has opposite effects on two hippocampus-dependent behavioral paradigms. One result that must be taken into consideration is the improved motor activity of TLR4-knockout mice. These mice exhibit a higher swimming speed in the Morris water maze and enhanced motor performance on the rotarod test[45]. The higher locomotor activity of TLR4-knockout mice may account for the reduced freezing response rate in the fear conditioning task. Thus, it is difficult to conclude whether TLR4 deficiency indeed impairs contextual fear conditioning. Another spatial learning/memory paradigm that is less sensitive to locomotor activity is needed to further evaluate the function of TLR4 in hippocampus-dependent learning and memory.

Although the role of TLR9 in neurodevelopment and neurodegeneration has yet to be investigated, the behaviors of TLR9-knockout mice have been described[46]. In contrast to TLR3- and TLR4-knockout mice, TLR9-knockout mice do not exhibit any phenotype in the Morris water maze. However, they exhibit hyperactive sensory responses and motor behaviors. TLR9 mutant mice are more sensitive to thermal stimuli in response to a hot plate. Moreover, motor responsiveness under anxiety-provoking conditions in an open field test is enhanced in these mice; similarly, prepulse inhibition of the acoustic startle response is also enhanced[46]. This study indicates that TLR9 is important for sensory and motor behaviors in mice.

Interestingly, although TLRs share similar signaling pathways and downstream mediators to trigger innate immune responses, the behavioral phenotypes of TLR3-, TLR4- and TLR9-deficient mice are distinct. These findings suggest that each TLR likely performs a unique function in the brain. It is not clear whether these distinctions are related to the expression levels or patterns of these TLRs in the brain. It is also possible that TLRs use unique downstream pathways in neurons, thus resulting in distinct functions in the brain. The detailed signaling pathway of each TLR in neurons needs to be investigated to address this possibility.

Notably, all of the knockout mice used in the studies discussed above are conventional knockout mice. Thus, TLRs are missing from both the nervous system and peripheral tissue. Although neuronal TLRs have been shown to regulate neurodevelopment and neurodegeneration, it cannot be ruled out that TLRs in peripheral tissues may indirectly influence brain function by modulating peripheral innate immunity. Neuron-specific knockout mice are required to conclusively determine the roles of neuronal TLRs in cognition and behaviors.

Function of Sarm1 in Brain

The predominantly neuronal expression of Sarm1 distinguishes it from all other TIR domain-containing adaptors involved in TLR signaling[12, 14]. Consistent with the original finding regarding the involvement of Sarm1 in innate immunity[47], Sarm1 knockdown in the mouse brain disrupts the expression levels of inflammatory and antiviral cytokines. At the embryonic stage, Sarm1 knockdown increases IL-6 and IFNβ expression. In the adult Sarm1 knockdown brain, IL-1β, IL-12 and CCL5 are upregulated, while TNFα and IFNβ are downregulated[13]. Interestingly, Sarm1 is only expressed in neurons but not glia in the brain[13]. The aberrant cytokine expression profiles found in Sarm1-knockdown brains suggest the critical role of

neurons in controlling innate immune responses in the brain.

Similar to TLR3 and TLR7, Sarm1 also controls neuronal morphology and function. The first study revealing the role of Sarm1 in neurons used *Caenorhabditis elegans*. Tir-1, the *C. elegans* ortholog of Sarm1, is critical for synaptic signaling to the nucleus, and is involved in the left-right asymmetric expression of the odorant receptor in olfactory neurons[48]. Tir-1 receives a Ca^{2+} signal *via* CaMK and transduces its signals *via* the ASK1-MEK4/7-JNK pathway consequently regulating gene expression[48]. The synaptic localization of Tir-1 is microtubule-dependent[49]. Because Tir-1 influences the JNK complex in *C. elegans* neurons, the effect of Sarm1 on JNK was also investigated in mammalian neurons. The data indicate that Sarm1 associates with JNK3, recruits JNK3 to mitochondria, and regulates cell death after deprivation of glucose and oxygen[14]. This discovery has recently been confirmed by other studies[50, 51].

In addition to cell death, Sarm1 is actually important for neuronal morphology. Using GST-syndecan-2 fusion protein as bait, Sarm1 has been identified as a novel syndecan-2-interacting protein in the mouse brain[12]. Syndecan-2, a synaptic heparan sulfate proteoglycan, regulates synapse formation and dendritic arborization through various downstream mediators[12, 52]. For syndecan-2-mediated synapse formation and maintenance, both neurofibromin and CASK protein complexes are required[52-54]. Sarm1 is essential for syndecan-2-regulated dendritic arborization. Sarm1 receives signals from synaptic syndecan-2 and acts through the ASK1-MEK4/7-JNK pathway to modulate dendritic arborization[12]. Interestingly, Sarm1 expression in neurons is detectable far earlier than that of syndecan-2. Therefore, Sarm1 also controls syndecan-2-independent events, such as axonal outgrowth and the establishment of neuronal polarity[12]. Thus far, the upstream signal of Sarm1 in regulating axonal outgrowth and neuronal polarity is still unclear. Since Sarm1 functions as an adaptor molecule, the identification of additional Sarm1-interacting proteins would provide clues to the mechanism underlying its role in axonal outgrowth and neuronal polarity.

Sarm1 is also critical for axon degeneration during injury. In both flies and mice, deletion of Sarm1 effectively prevents Wallerian degeneration for weeks after axotomy[55]. The TIR domain of Sarm1 is important for activation of the downstream destruction pathway, while multimerization mediated by the SAM domain of Sarm1 is also essential for the function of Sarm1 to trigger axon degeneration[56]. Despite the involvement of its SAM and TIR domains, it is completely unclear which pathway Sarm1 uses to trigger axon degeneration. Association with mitochondria is clearly not required for Sarm1-dependent axon degeneration[56]. There is also no evidence regarding whether the ASK1-MEK4/7-JNK pathway plays a role in Sarm1-mediated axon degeneration. It is puzzling that Sarm1 appears to play both positive and negative roles in neuronal morphology. Sarm1 is required for neuronal morphogenesis during development, but it triggers axon degeneration after injury. Because Sarm1 is widely distributed throughout the various subcellular compartments of neurons, it is possible that it associates with various proteins at distinct subcellular regions consequently regulating different events. The studies of the functions of Sarm1 are summarized in Figure 3.

The influence of Sarm1 on neurons also results in abnormal behaviors and electrophysiological responses in Sarm1-knockdown mice[57]. Although Sarm1-deficient mice show normal locomotor activity and anxiety behaviors, they exhibit several autism-like behaviors, including reduced cognitive flexibility and greatly decreased social interactions. Besides, Sarm1-knockdown transgenic mice are defective in both contextual and auditory fear conditioning[57]. Echoing the defects in associative memory, these mice have hyper-NMDAR-dependent long-term potentiation and impaired mGluR-dependent long-term depression (LTD)[58]. Treatment with CDPPB, a positive mGluR allosteric modulator, effectively ameliorates the mGluR-dependent LTD, associative memory, and social interaction[58]. Because Sarm1 regulates neuronal innate immunity, morphogenesis, and activation, the behavioral defects of Sarm1-knockdown mice support the hypothesis that immune challenge during early development increases the risk of psychiatric disorders later on. Although direct evidence of mutations in the human Sarm1 gene in patients suffering from psychiatric disorders is lacking, several independent studies suggest an association of Sarm1 with autism spectrum disorders. First of all, the human Sarm1 gene is located at chromosome 17q11 (17:26,698,987–26,728,065), which is within the locus

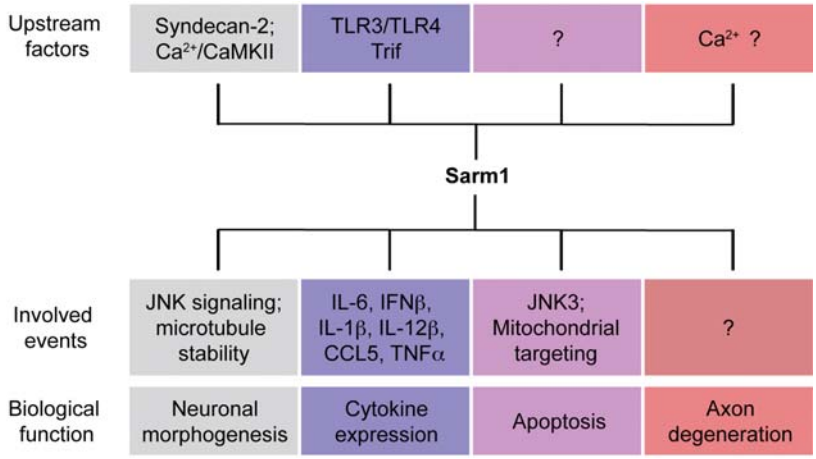

Fig. 3. Summary of Sarm1 function in neurons. See detailed description in the main text.

of autism, susceptibility to, 6 (Auts6, OMIM%609378, 17:24,000,000–31,800,000). Second, a comparison of the protein expression profiles of control individuals and patients suffering from autism revealed that the Sarm1 protein levels in the mid-frontal cortex are decreased in autistic patients[59]. Finally, Sarm1 mRNA has been predicted to be recognized by Fragile X mental retardation protein (FMRP)[60], which is encoded by the Fragile X mental retardation 1 gene. Because Fragile X syndrome is a well-known monogenic disorder associated with autism, recognition of Sarm1 by FMRP also implies an association of Sarm1 with autism. It is likely that Sarm1 plays a critical role in linking the innate immune response to neuronal morphogenesis and psychiatric disorders.

Conclusion

Neuronal TLRs and TIR domain-containing adaptor molecules not only regulate the innate immune responses of neurons but also play critical roles in controlling neuronal morphogenesis and function. These new findings impact the hypotheses regarding crosstalk between the nervous and immune systems. Although the detailed signaling pathways and the molecular regulation of TLRs and TIR domain-containing adaptors in neurons are largely unknown, the cell-autonomous innate immune responses likely play crucial roles. Neuron-specific knockout mice should be used in the future to further evaluate the contribution of neuronal innate immune responses to the regulation of neuronal development, degeneration, and function.

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