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Full-Length Review

Toll-like receptors in central nervous system injury and disease: A focus on the spinal cord

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ABSTRACT

Toll-like receptors (TLRs) are best known for recognizing pathogens and initiating an innate immune response to protect the host. However, they also detect tissue damage and induce sterile inflammation upon the binding of endogenous ligands released by stressed or injured cells. In addition to immune system-related cells, TLRs have been identified in central nervous system (CNS) neurons and glial subtypes including microglia, astrocytes and oligodendrocytes. Direct and indirect effects of TLR ligands on neurons and glial subtypes have been documented *in vitro*. Likewise, the effects of TLR ligands have been demonstrated *in vivo* using animal models of CNS trauma and disease including spinal cord injury (SCI), amyotrophic lateral sclerosis (ALS) and neuropathic pain. The indirect effects are most likely mediated via microglia or immune system cells that infiltrate the diseased or injured CNS. Despite considerable progress over the past decade, the role of TLRs in the physiological and pathological function of the spinal cord remains inadequately defined. Published reports collectively highlight TLRs as promising targets for therapeutic interventions in spinal cord pathology. The findings also underscore the complexity of TLR-mediated mechanisms and the necessity for further research in this field. The goals of the current review are to recapitulate the studies that investigated the role of TLRs in the spinal cord, to discuss potential future research directions, and to examine some of the challenges associated with pre-clinical studies pertinent to TLRs in the injured or diseased spinal cord.

1. Introduction

Toll-like receptors (TLRs) are the mammalian homologues of the *Drosophila melanogaster* Toll (Medzhitov et al., 1997), a transmembrane receptor (Hashimoto et al., 1988) that plays a crucial role in mediating *Drosophila* immunity (Halfon et al., 1995; Lemaitre et al., 1996; Qiu et al., 1998) as well as embryogenesis, particularly in the establishment of the dorsal–ventral axis and motoneuron development (Anderson et al., 1985a,b). Toll-like receptors are one of several classes of pattern-recognition receptors (PRRs), which are collectively known for their role in the activation of the innate immune system and the subsequent orchestration of the adaptive immune response (for reviews see Kaisho and Akira, 2006; Medzhitov and Janeway, 2000). Upon recognition of conserved molecular motifs expressed by pathogens, referred to as "pathogen-associated molecular patterns" (PAMPs), TLRs initiate a cascade of intracellular events involving the Nuclear Factor-kappa B (NF-κB)-dependent production and release of cytokines and chemokines (Hirschfeld et al., 1999; Medzhitov et al., 1997). PAMPs consist of signature sequences crucial for pathogen survival in the host and comprise all macromolecular classes including lipopolysaccharide (LPS), a component of the cell wall of gram negative bacteria (Poltorak et al., 1998a), peptidoglycans derived from gram positive bacterial cell walls (Takeuchi et al., 1999a), flagellin (Hayashi et al., 2001), lipoproteins (Brightbill et al., 1999), as well as single stranded RNA (Diebold et al., 2004; Heil et al., 2004) or double stranded RNA (Alexopoulou et al., 2001; Liu et al., 2008) and unmethylated CpG DNA (Hemmi et al., 2000; Latz et al., 2007).

Thus far, TLRs 1–10 have been identified in humans (Chaudhary et al., 1998; Chuang and Ulevitch, 2000, 2001; Rock et al., 1998; Takeuchi et al., 1999b) with TLR11 rendered non-functional by the presence of a stop codon (Zhang et al., 2004). TLRs 1–9 and TLR 11–13 have been identified in mice (Du et al., 2000; Hemmi et al., 2000; Poltorak et al., 1998a,b; Sebastiani et al., 2000; Shi

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et al., 2011a; Takeuchi et al., 1999b) with TLR10 rendered nonfunctional by a retroviral insertion (Hasan et al., 2005). TLRs 1, 2, 4–6, 10–12 are located on the cell surface whereas TLRs 3, 7–9, and 13 are localized to the membranes of endosomal compartments and the endoplasmic reticulum in immune system-related cells including polymorphonuclear leukocytes (PMNs; Muzio et al., 2000), monocytes (Kadowaki et al., 2001; Sabroe et al., 2002), dendritic cells (Kadowaki et al., 2001), T lymphocytes (Muzio et al., 2000) and B lymphocytes (Bourke et al., 2003; Hornung et al., 2002). However, evidence indicates that TLRs are also expressed in the human (Bsibsi et al., 2002) and rodent (Laflamme et al., 2001) central nervous system (CNS) cells, including microglia (Bsibsi et al., 2002; Kigerl et al., 2007), oligodendrocytes (Bsibsi et al., 2002; Kigerl et al., 2007), astrocytes (Bsibsi et al., 2002; Bowman et al., 2003; Kigerl et al., 2007) and neurons (David et al., 2013; Lafon et al., 2006; Ma et al., 2006).

Importantly, TLRs also play a role in non-infectious conditions associated with tissue insult and repair wherein endogenous ligands known as "damage-associated molecular patterns" (DAMPs) may be released in response to cellular stress, injury or death. DAMPs can bind and trigger TLR activation and consequently contribute to sterile inflammation. Some DAMPs are confined to the intracellular space under physiological conditions and are released into the extracellular space following injury- or disease-induced cellular damage and death. These DAMPs include high mobility group box 1 (HMGB-1) (Park et al., 2004), heat shock proteins (HSPs) (Asea et al., 2002; Ohashi et al., 2000), microRNA (Lehmann et al., 2012), mitochondrial RNA and DNA (Kariko et al., 2005; Zhang et al., 2010) and histones (Huang et al., 2011). In addition, stressed and injured cells release activated proteases which degrade the extracellular matrix, and thereby, generate additional DAMPs including hyaluronic acid (Termeer et al., 2002), fibrinogen (Smiley et al., 2001), fibronectin (Gondokaryono et al., 2007) and biglycan (Schaefer et al., 2005).

TLRs have been implicated in neuroinflammation associated with a number of neurological and neurodegenerative conditions of the CNS. Depending on the cell type and the condition under which the receptor is activated, both detrimental and beneficial roles have been attributed. The goal of this review is to highlight the expression and the roles of TLRs in the healthy spinal cord and in pathological conditions that affect the spinal cord including traumatic injury, neuropathic pain and amyotrophic lateral sclerosis (ALS).

2. Overview of toll-like receptor signaling

TLRs are type I transmembrane glycoproteins containing a leucine-rich repeat (LRR; Hashimoto et al., 1988) motif on the extracellular domain that mediates ligand binding, and a toll-interleukin-1 (IL-1) receptor (TIR) intracellular domain (Rock et al., 1998) that facilitates the binding of downstream adaptor proteins (Medzhitov et al., 1998; Muzio et al., 1998; Takeuchi et al., 2000). Following ligand binding, TLRs form homodimers (Bovijn et al., 2012) or heterodimers (Hajjar et al., 2001; Ozinsky et al., 2000a,b) and initiate signaling through either the myeloid differentiation 88 (MyD88)-dependent or MyD88-independent pathways (Akira and Takeda, 2004). Toll-like receptors 1–2 and 5–13 signal primarily through the MyD88 protein. In addition, TLR2 uses the bridging adaptor toll-interleukin 1 receptor domain containing adaptor protein (TIRAP) to recruit MyD88 to its TIR domain (Yamamoto et al., 2002). TLRs that primarily utilize the MyD88-dependent pathway can activate the transcription factors activator protein (AP)-1 (Chiu et al., 2009; Liu et al., 2009a) via the p38/c-Jun N-terminal kinase (JNK)/extracellular signal regulated kinase (ERK) pathway (An et al., 2002), Nuclear Factor-κB (NF-κB; Zhang et al., 1999) or interferon regulatory factor 5 (IRF5; Takaoka et al., 2005). Translocation of these transcription factors to the nucleus promotes the transcription of inflammatory cytokines.

Toll-like receptor 3 signals exclusively through the MyD88-independent, TIR-domain-containing adaptor protein inducing IFN-β (TRIF) pathway which drives the activation of interferon regulatory factor 3 (IRF3; Sato et al., 2003) and IRF7 (Fitzgerald et al., 2003; Han et al., 2004), resulting in transcription of type I interferons (IFNs; Sato et al., 1998a,b). In addition, TLR3 can activate the transcription factors IRF5 (Barnes et al., 2001; Takaoka et al., 2005), AP-1 (Chiu et al., 2009; Liu et al., 2009a) and NF-κB through the adaptor protein TNF receptor associated factor 6 (TRAF6) leading to transcription of inflammatory cytokines (Mukundan et al., 2005).

TLR4 is unique as it can signal through both MyD88 (Kawai et al., 1999; Takeuchi et al., 2000) and TRIF signaling pathways (Fitzgerald et al., 2003; Yamamoto et al., 2003). Some of the major TLR signaling pathways have been illustrated in Fig. 1 and the current knowledge about TLR signaling has been discussed, in detail, in a recent review (Sasai and Yamamoto, 2013).

3. Inflammation and tissue damage elicited by intrathecal or intraspinal delivery of TLR agonists to naïve rodents

Toll-like receptors 1–10 are present in the healthy adult human spinal cord with TLR2, TLR4, TLR6, and TLR7 showing the highest expression at the mRNA level (Nishimura and Naito, 2005). TLR mRNA (Adhikary et al., 2011; Kigerl et al., 2007) or protein (David et al., 2013) has also been detected in the rodent spinal cord.

A number of studies investigated the molecular and cellular responses in the spinal cord following intrathecal (i.t.) or intraspinal (direct injection into the spinal cord parenchyma) delivery of TLR ligands to naïve rodents. Intraspinal injection of zymosan, a TLR2 agonist, activated resident microglia and induced infiltration of monocytes and blood-derived macrophages, which was paralleled by demyelination, axonal injury, and astroglial activation (Popovich et al., 2002). However, despite these histopathological changes, only a small portion of the injected rats developed transient anomalies in open field locomotor function (Popovich et al., 2002). The detrimental effects of intraspinal zymosan on myelin and axonal integrity have been attributed to TLR2-mediated activation of macrophages (Schonberg et al., 2007).

Whereas the studies of Schonberg et al. (2007) on the spinal cord suggested that zymosan reduces myelin integrity via the release of toxic effectors by inflammatory cells, investigations on cultured brain oligodendrocytes and oligodendrocyte progenitor cells (OPCs) indicated that zymosan can have direct and beneficial effects on these cells (Bsibsi et al., 2012). Zymosan promoted the survival and differentiation of brain OPCs and immature oligodendrocytes, *in vitro*, without altering the survival of mature oligodendrocytes (Bsibsi et al., 2012). In contrast to zymosan, hyaluronan, another TLR2 agonist, blocked the maturation of brain OPCs, *in vitro* (Sloane et al., 2010). The presence of TLR2 immunoreactivity in oligodendrocytes of normal and pathological human brain tissue has been reported (Bsibsi et al., 2002; Sloane et al., 2010) and supports the notion that TLR2 ligands could directly act on cells of the oligodendrocyte lineage, not only *in vitro* but also *in vivo*.

Based on the aforementioned investigations, we postulate that zymosan could have divergent effects on cells of the oligodendrocyte lineage. Such effects could be mediated through direct as opposed to indirect mechanisms. It is possible that the direct and beneficial effects of zymosan on OPCs and immature oligodendrocytes are detectable only when the cells are isolated from their

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