Neuroinflammation Mini Review
The role of TLRs in neuroinflammation

Mini Review

Neuroscience

Neurodegenerative diseases affect millions of people all over the world; for example over 5 million people in the United States alone have been diagnosed with Alzheimer's disease (AD). Although all underlying causes are not fully understood, neuroinflammation and immune responses play a big part in the onset of neurodegeneration. Evidence shows that microglia, astrocytes, oligodendrocytes and neurons are all capable of initiating immune responses in the brain that could contribute to AD as they produce and signal via neuroinflammatory components such as cytokines, interleukins, chemokines, leukocytes and endothelial adhesion molecules. In that context much research focuses on decyphering the role played by reactive oxygen species, Toll like receptor (TLR) and NFkappaB (NFκB) mediated signaling pathways.

What is neuroinflammation?

Is neuroinflammation a cause or a consequence of neurodegeneration and is it protective or destructive? Acute neuroinflammation is required for the removal of amyloid beta (Aβ) plaques by microglia. On the other hand on-going neuroinflammation results in glial dysfunction and neuronal compromise. Therefore, the time period and intensity of the inflammatory response is critical in regard to answering whether the response is protective or destructive.

Injury to the brain leads to the activation of repair pathways such as the recovery of the neuronal network, enhancing plasticity and providing alternative methods of performing tasks that were abolished due to the loss of brain tissue. Neuroinflammation is the response by glial cells (e.g. microglia and astrocytes) to infection or injury as part of the innate immune system. Activated glial cells produce pro-inflammatory cytokines, enzymes and adhesion molecules - a response aided by TLRs.

Ischemic mice negative for tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6), showed a larger amount of necrotic tissue when their inflammatory response was activated. This indicated a protective role of the inflammation mediator products TNFα and IL-6. In addition to that, other cytokines such as the IL-1 family play a detrimental role in inflammation e.g. the deletion of IL-1α and IL-1β in mice reduced ischemic brain damage (Boutin et al. 2001). IL-1 is expressed after insult and in an NF-κB dependent manner. It therefore appears that timing as well as specific cytokine expression, which is often regulated by TLR signaling, is crucial in determining whether inflammation helps or hinders repair.

Toll like receptors and aging

During aging secretion of pro-inflammatory cytokines is increased, whereas levels of anti-inflammatory cytokines decrease. Similarly TLR signaling is also altered during aging. This suggests that TLRs and inflammation are important factors in the onset of all kinds of age-related diseases.

What are toll like receptors?

TLRs play a key role in the development and regulation of inflammation, neurodegeneration and brain trauma. They are crucial components in activating the innate immune response against invading pathogens in addition to playing a key role in neuronal degeneration, neurogenesis and neurite outgrowth. All these aspects taken together suggest an important role in maintaining neuronal plasticity. In total eleven human and thirteen mouse TLRs have been identified. All belong to the pattern recognition receptor (PRR) family, along with other receptors such as CD206, and NALP (a Nod-like receptor). TLRs are expressed on a large variety of immune-related cells, including microglia, astrocytes, oligodendrocytes and neurons. Their expression patterns are dependent on the presence of pathogens, cytokines and environmental stresses.

TLRs are either expressed on the cell surface (TLR1, 2, 4, 5 and 6) or in endosomal vesicles (TLR3, 7, 8 and 9). Characteristic for all TLRs is a leucine-rich extracellular domain, which is involved in recognizing pathogen associated molecular patterns (PAMPs). Also conserved is a cytoplasmic Toll-IL-1 receptor (TIR) domain, which initiates downstream signaling via mediating interactions between TLRs and TIR-adapter proteins such as myeloid differentiation factor 88 (MyD88), TIR-adapter proteins (TIRAP), TIR-domain-containing adapter-inducing interferon-β (TRIF) and TRIF-related adaptor molecule (TRAM). These interactions result in the upregulation and expression of inflammation regulating transcription factors such as NF-κB.

TLR4 is highly conserved from *Drosophila melanogaster* to humans. The active form of TLR4 is able to activate the NF-κB pathway thereby leading to the expression of NF-κB-dependent inflammatory genes and CD80. These are all necessary for the activation of naïve T lymphocytes, key players in an innate immune response (Medzhitov et al. 1997). Repeated stress exposure upregulates TLR4 expression suggesting a key role of TLR4 in stress-induced NF-κB activation, iNOS and COX-2 upregulation, and cellular oxidative/nitrosative damage responses. TLR4 signaling also affects IL-1β, IL-10 and IL-7 levels.

TLRs form either homo- or heterodimers and are categorized into three main groups (Table 1) according to the specific PAMP motif they recognize. TLR classes are:

1. TLRs recognizing lipids

2. TLRs activated by protein ligation

3. TLRs recognizing bacterial and viral nucleic acids.

Location	Group	TLR	Dimerizes with	Agonists
Cell surface	Activated by lipids	TLR1 (CD281)	TLR1	
		TLR2	CD36	
		TLR2 (CD282)	TLR1	Pam3Cys (bacterial peptide) triacylated lipopeptides
		TLR2	TLR2	Heat shock proteins
		TLR2	TLR6	Diacylated lipopeptides (Pam2CSK4), peptidoglycan
		TLR4 (CD284)	TLR4	Lipopolysaccharide (LPS) (Gram negative bacteria/bacteria flagellin), heat shock proteins
		TLR6 (CD286)	TLR6	
		TLR10 (only in humans)	TLR 1/2	
	Activated by protein ligation	TLR5	TLR5	Bacterial flagellin proteins
		TLR11	TLR11	Unknown ligand of bacteria and *Toxoplasma Gondii*
Endosomal vesicles	Activated by bacterial and viral nucleic acids	TLR3 (CD283)	TLR3	Double stranded viral RNA
		TLR7 (murine homolog of TLR8)	TLR7	Imidazoquinoline and viral ssRNA
		TLR8 (CD288)	TLR8	
		TLR9 (CD289)	TLR9	Unmethylated CpG dinucleotides

Table 1: TLR Overview

The TLR pathway

After PAMP recognition, TLR signaling is mediated in either a MyD88 dependent or MyD88 independent/TRIF dependent manner. MyD88 is an adapter protein that mainly interacts with TIRAP/MyD88-adapter-like (Mal) and TRAM proteins and plays a crucial role in enabling the recruitment of the IL-1R associated kinases (IRAK) 1, 2, 3 and 4. TIRAP/Mal and TRAM are also required for the activation of TRIF dependent signaling. All TLRs signal via the MyD88-dependent pathway, except for TLR3, which signals exclusively via TRIF.

Key steps in both MyD88 and TRIF mediated TLR signaling have been outlined below using TLR4 as an example. The outcome of both pathways is the activation of IRF3, NFκB and AB-1, which regulate the expression of pro-inflammatory cytokines and type 1 IFN.

MyD88 dependent pathway

1. TLR4 is stimulated by ligand binding
2. MyD88 is recruited via TIRAP
3. MyD88 recruits IRAK kinases (mainly IRAK4)
4. IRAKs are phosphorylated and subsequently activated
5. IRAKs dissociate from MyD88
6. IRAKs interact with the E3 ligase TRAF6
7. TRAF6 forms a complex with the ubiquitin conjugating enzymes UBE2N/UBC13 and UBE2V1/UEV1A. This complex polyubiquitinates target proteins such as TAK1/TAB1/TAB2/TAB3
8. Polyubiquitination leads to TAK complex activation and subsequent IKK activation
9. IkappaBs are destroyed by the 26S proteasome resulting in translocation of NF-κB to the nucleus and regulation of NF-κB dependent genes
10. MAP kinase signaling is also activated by the TAK complex resulting in the induction of pro-inflammatory cytokines through AP-1 activation.

MyD88 independent/TRIF dependent pathway

Pathway 1

1. TLR4 is stimulated by ligand binding
2. TLR4 recruits TRAM and TRIF
3. TRIF interacts with TBK1
4. TBK1 kinase and IKKi enable IRF3 phosphorylation
5. IRF3 phosphorylation results in its activation and translocation into the nucleus where it works as a transcription factor

Pathway 2

1. TLR4 is stimulated by ligand binding
2. TRIF interacts with TRAF6 and RIP1
3. This results in destruction of IkappaBs by the 26S proteasome and subsequent NF-κB activation

TLRs and brain cells

Microglia

Microglia are macrophage-like cells present in the central nervous system (CNS) that mediate neuronal immune interactions. Activated microglia appear to have mainly a neuroprotective role in acute injury. Stress conditions, such as hypoxia, result in increased TLR signaling in microglia. Apart from TLR10, all TLRs are expressed in microglia (Table 2). TLR4 and TLR2 for example have been implicated in sensitizing microglial apoptosis suggesting a role in preventing excessive inflammation. Microglia produce pro-inflammatory cytokines, enzymes and adhesion molecules, which initiate leukocyte migration through the blood brain barrier and promote effector functions in these infiltrating cells.

TLRs in microglia	Agonists	Outcome	Comments
TLR2	LPS, gram positive and negative bacteria	IFNβ	Decreases MHC II and CD4 T cell proliferation
		IL-6, IL-10	Initial microglial response to axonal injury
	Group B Streptococci		Causes microglial apoptosis
TLR3	Poly I: C	CXCL-10, IFNβ, IL-6, IL-10, IL-12, TNFα,	Induces TH1 polarization and IFNγ secretion of CD4 T cells
TLR4	LPS	IFNβ	Increases apoptosis in microglia via IFNβ production
		IL-1β, IL-6, IL-7, IL-10, and TNFα	Decreases MHC II and CD4 T cell proliferation
		NF-κB independent/ JNK-mediated GDNF gene expression	Neuroprotective role

Table 2: Role of TLRs in microglia

bioNova científica, s.l. · Tel.: 915 515 403 · Fax: 914 334 545 · e-mail: tec@bionova.es · www.bionova.es

Astrocytes

Human astrocytes express TLR1-7, 9 and 10 (Table 3). Drugs such as statins increase astrocyte TLR4-mediated cytokine production and are linked to neuropathies. Cytokines and TLR agonists in return also increase the expression of chemokine ligands such as CCL2, CCL3, and CCL5, which go on to attract immune cells to the site of inflammation.

TLRs in astrocytes	Agonists	Outcome	Comments
TLR2	CD14	CXCL8, IL-6, IL-12, IL-12p40	
	LPS	IFNβ, TNFα	
TLR3	Poly I: C	CXCL10, IFNα4, IFNβ, IL-6, iNOS, TNFα	These products lead to growth inhibition of astrocytes, endothelial cells and survival of neurons suggesting a neuroprotective role
TLR4	LPS	IFNα4, IFNβ, IL-6, iNOS, TNFα	
	LPS and dsRNA	GM-CSF, IL-1α, IL-6, L-1α, LTB, TNFα, TGF-B3	

Table 3: Role of TLRs in astrocytes

Oligodendrocytes

Oligodendrocytes are a type of neuroglia that support and insulate axons in the CNS by creating a myelin sheath. Injury to these cells can result in demyelination, and subsequently development of diseases such as Multiple Sclerosis. Little evidence is available on the role of TLRs in oligodendrocytes, apart from the fact that TLR2, 3 and 4 are expressed in these cells.

Oligodendrocytes, in which TLR signaling has been activated by treatment with the TLR2 agonist Zymosan, have been shown to play a role in the repair of damage to the CNS and inflammation-mediated remyelination (Setzu et al. 2006). This strongly suggests a neuroprotective role of TLR2. However, contradicting results imply that Zymosan-mediated TLR2 activation results in complete oligodendrocyte loss and demyelination of intact myelin axons around lesions (Schonberg et al. 2007). Further research is needed to fully understand the role of TLR2.

Neurons

There is increasing evidence for the importance of aberrant TLR neuronal expression in the development of pathological conditions. A distinct difference is seen in TLR activation in differentiated neurons versus neuronal progenitor cells, although both cell types express TLR2 and TLR4. TLR2 is involved in hippocampal neurogenesis, while TLR4 reduces proliferation and neuronal differentiation. Both TLRs regulate cell fate via MyD88 and NF-κB pathways, however, this is specific to progenitor cells, the mediators of differentiated cells are unknown.

TLRs in neurons	Location	Agonists	Comments
TLR2 deficiency	Adult neuronal progenitor cells		Impairs hippocampal neurogenesis
TLR3	Growth cones of neurons	Poly I: C	Causes collapse of growth cones, inhibits neurite extension of cortical and hippocampal neurons, reduces proliferating cells and neurosphere formation
TLR4	Nociceptive neurons	LPS and CD14	
TLR4 deficiency	Adult neuronal progenitor cells		Increases proliferation and neuronal differentiation
TLR8		R848	Inhibits neurite outgrowth and induces neuronal apoptosis

Table 4: Role of TLRs in Neurons

bioNova científica, s.l. · Tel.: 915 515 403 · Fax: 914 334 545 · e-mail: tec@bionova.es · www.bionova.es

Conclusion

This minireview has emphasized the importance that TLRs play in the mediation and regulation of neuroinflammation. Although a lot of progress has been made in researching neuroinflammation, especially in a disease context, many questions remain open. These include why different neuronal cell types show different levels of damage vulnerability and identifying the molecular mechanisms underlying inflammatory neurodegeneration. Additionally it is essential to understand the control of recovery and neuronal plasticity after inflammation induced brain damage.

References

Boutin H et al. (2001). Role of IL-1alpha and IL-1beta in ischemic brain damage. **J Neurosci 21, 17.**

Medzhitov R et al. (1997). A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. **Nature 388, 394-397.**

Okun E et al. (2009). Toll-like Receptors in Neurodegeneration. **Brain Res Rev 59, (2) 278-292.**

Schonberg DL et al. (2007). Oligodendrocyte generation is differentially influenced by toll-like receptor (TLR) 2 and TLR4-mediated intraspinal macrophage activation. **J Neuropathol Exp Neurol 66, 1124-1135.**

Setzu A et al. (2006). Inflammation stimulates myelination by transplanted oligodendrocyte precursor cells. **Glia 54, 297-303.**

Tel.: 915 515 403
Fax: 914 334 545
e-mail: info@bionova.es

www.bionova.es