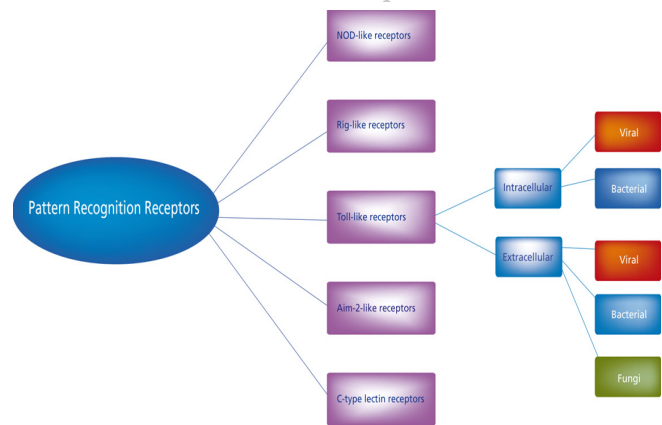


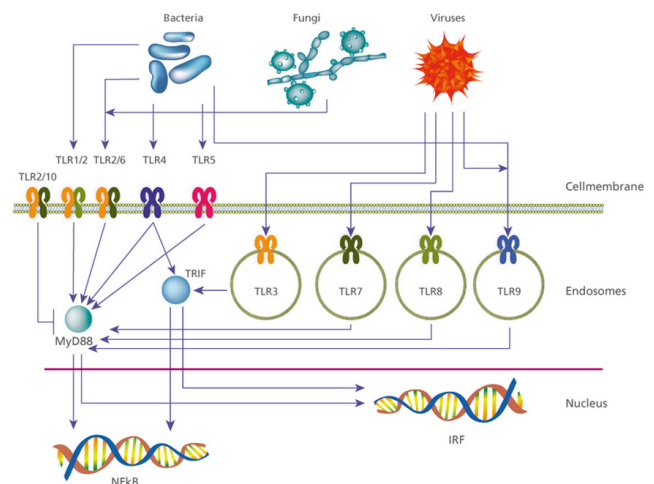


to the pattern recognition receptors [Figure 1]. They can recognize both small molecular motifs conserved across microbes (pathogen-associated molecular pattern or PAMP) [Figure 2], and endogenous molecules generated during inflammation or tissue damage (damage associated molecular pattern or DAMP).<sup>[2-5]</sup> TLRs can initiate an acute inflammatory reaction and subsequently can coordinate the activation of the adaptive immune system. To date, thirteen TLRs are known, of which ten (TLR1-10) have been described in humans.<sup>[6]</sup> The cell surface TLRs recognize PAMPs that are mainly constituent of the bacterial cell wall or are expressed on the bacterial cell surface, such as lipopeptides and peptidoglycan (TLR1/TLR2, TLR2/TLR6, TLR2/TLR10), lipopolysaccharide (LPS) (TLR4) and flagellin (TLR5). In contrast, the intracellular TLRs mainly recognize microbial nucleic acid including viral double-strand RNAs (TLR3), single-strand RNAs (TLR7 and TLR8) and CpG ODN (TLR9).<sup>[7]</sup> TLRs can employ two second messenger pathways; the myeloid differentiation primary response gene 88 (MyD88) pathway, activating nuclear factor  $\kappa$ -light-chain-enhancer of activated B cells (NF- $\kappa$ B), or the TIR-domain-containing adapter-inducing interferon- $\beta$  (TRIF) pathway, activating interferon regulatory factor 3 (IRF3) [Figure 2]. NF- $\kappa$ B controls DNA transcription resulting in the production of pro-inflammatory cytokines such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), interleukin (IL)1 $\beta$  and IL6.<sup>[7,8]</sup> IRF3 is an interferon (IFN) regulatory factor leading to the production of antiviral type I IFN.<sup>[2,7]</sup>

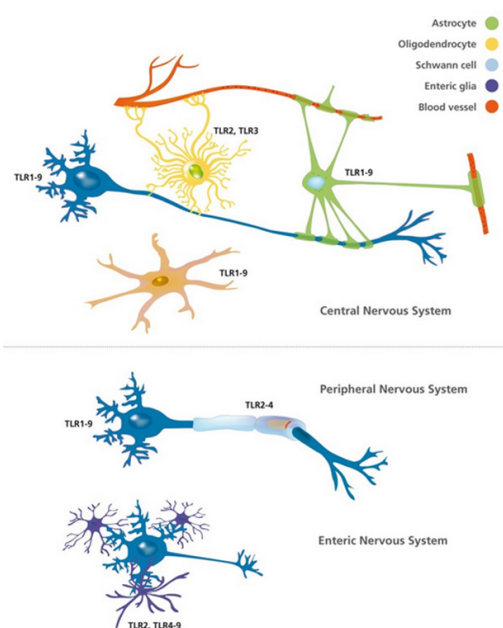
The presence of TLRs on immune cells and epithelial cells is well known, but their expression is not restricted to these cell types. Glial cells and neurons express TLRs in both the peripheral nervous system (PNS) and the central nervous system (CNS) [Figure 3], allowing neurons to act as immune cells.<sup>[9-15]</sup> More specifically, in the CNS neurons, astrocytes and microglial cells express TLR1-9, whereas oligodendrocytes express only TLR2 and TLR3.<sup>[16-20]</sup> Peripheral neurons also express TLR1-9 and enteric glial cells express TLR1-5, TLR7 and TLR9.<sup>[13,14,21-23]</sup> Neuronal TLR signaling pathways do not necessarily employ NF- $\kappa$ B<sup>[24-26]</sup> and may involve the glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ), jun-N-terminal kinase (JNK) and phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathways.<sup>[27-29]</sup> Interaction between neurons and the immune system has already been reported, setting the scene for neurons acting as immune cells.<sup>[30-34]</sup> It has been reported that neuronal TLRs are involved in the development and homeostasis of the nervous system, and notably in several neurodegenerative diseases.<sup>[35,36]</sup> Both TLR2 and TLR4 are involved in neuronal apoptosis, development and survival in the context



**Figure 1:** Toll like receptors are part of the innate immune system and belong to the pattern recognition receptors



**Figure 2:** Different pathogens activate different TLRs. TLRs signal through two different pathways using myeloid differentiation primary response gene 88 (MyD88) and TIR-domain-containing adapter-inducing interferon  $\beta$ , leading to activation of NF- $\kappa$ B and IRF respectively. NF- $\kappa$ B leads to DNA transcription and cytokine production, while IRF leads to interferon production. TLRs: toll like receptors; NF- $\kappa$ B: nuclear factor  $\kappa$ -light-chain-enhancer of activated B cells; IRF: interferon regulatory factor



**Figure 3:** TLRs are differentially expressed by neurons and glial cells of the central, peripheral and enteric nervous system. TLRs: toll like receptors





hippocampal neurons after I/R injury.<sup>[37]</sup> However, before suggesting a potential role of TLR2 in the treatment of stroke, clinical studies are needed to determine whether TLR2 is viable as a marker or target for treatment in stroke.

Animal models show that TLR2 is relevant in relation to stroke, however, they might obscure the specific importance of neuronal TLR2 in the context of glial cells. In order to isolate and study neuronal TLR2 in a stroke model, cultured neurons were exposed to glucose deprivation, a model of stroke.<sup>[29]</sup> Increased cell death was found in WT neurons, while TLR2<sup>-/-</sup> neurons were resistant to glucose deprivation induced cell death. In a neuronal cell line oxygen-glucose deprivation resulted in TLR2 upregulation and in an increase of non-apoptotic cell death.<sup>[85]</sup> These *in vitro* data confirm that stroke can result in neurodegeneration through the activation of neuronal TLR2, making neuronal TLR2 a potential player in brain damage after I/R injury in mice, independent from the influence of glial TLR2.

Information on TLR2 in the brain of patients with stroke is sorely missing and should be sought in future research, starting with investigating expression patterns in different brain regions. Also, a major focus of research should aim at distinguishing neuronal TLR2 from glial TLR2.

### TLR3

TLR3 recognizes double stranded RNA associated with viral infection, and host RNA. Ligand binding induces the production of anti-viral mediators like the type I interferons (IFNs), such as IFN- $\alpha$  and - $\beta$  production by leukocytes. These IFNs stimulate macrophages and natural killer cells to elicit an anti-viral response.<sup>[86]</sup>

TLR3 has not been studied in direct relationship to neurodegenerative diseases, but work has been performed on the effect of TLR3 in the development of the nervous system. TLR3 expression decreases in the embryonic CNS during neurogenesis.<sup>[58]</sup> Intrathecal injection of TLR3 agonist polyinosine: polycytidylic acid in postnatal day 4 mice resulted in sensory-motor deficits, neuroanatomical defects and fewer axons in the spinal cord, which was associated with neurodegeneration.<sup>[24]</sup> The role for TLR3 in this study was demonstrated by the fact that no anatomical or behavioral problems were found in TLR3<sup>-/-</sup> mice treated with polyinosine: polycytidylic acid.<sup>[24]</sup> It seems that TLR3 is involved in the proper development of the CNS in early fetal life, because the receptor is differentially expressed at different embryonic stages. After birth, stimulation of TLR3 results in neurodegeneration. The decrease in

expression of TLR3 during neurogenesis, as found in the embryonic brain, is also found in cultured neural progenitor cells (NPCs), making NPCs more sensitive to TLR3-mediated inhibition of proliferation than mature neurons.<sup>[58]</sup> Despite this reported decrease in TLR3 expression during neurogenesis, neurons do express functional TLR3.<sup>[24]</sup> In primary neurons, TLR3 stimulation inhibits neurite outgrowth and causes irreversible growth cone collapse, without affecting cell survival.<sup>[24]</sup> Different results were found in the high TLR3-expressing neuroblastoma cell line SK-N-AS, where exposure to a TLR3 ligand resulted in growth inhibition and apoptosis.<sup>[44]</sup> The difference in results on cell viability could be due to the use of different cell types (dorsal root ganglia,<sup>[24]</sup> NPCs,<sup>[58]</sup> and cell lines<sup>[44]</sup>), thus revealing the limits of cell culture as a model of biological processes.

Although all *in vivo* data are obtained from early life studies and interpretation of these data in the context of neurodegeneration must be done carefully, extrapolating these results leads to the hypothesis that stimulation of neuronal TLR3 could be detrimental in neurodegenerative diseases, especially in the context of viral infections. TLR3 is a viral sensing innate immune receptor. It is known that viral infections like influenza can cause neurodegeneration<sup>[87]</sup> and that viruses are linked to neurodegenerative diseases:<sup>[88]</sup> specifically hepatitis C virus, Epstein-Barr virus and human immunodeficiency virus (HIV) have been associated with PD.<sup>[89-91]</sup> The involvement of neuronal TLRs during viral infections is discussed in more detail in a later part of this review.

### TLR4

TLR4 detects LPS derived from Gram-negative bacteria and host-derived signaling molecules such as heat shock proteins, and extracellular matrix proteins, after which the innate immune system is activated, leading to an inflammatory response.<sup>[92-94]</sup>

#### PD

The expression of TLR4 is increased in PD and MSA post-mortem brain tissue, suggesting clinical relevance to TLR4 in PD and neurodegeneration in general.<sup>[50,74]</sup> Animal experiments have been used to further elucidate the role of TLR4 in PD. TLR4<sup>-/-</sup> mice were more vulnerable to dopaminergic neuronal loss and motor problems induced by  $\alpha$ -synuclein overexpression, but less vulnerable to the induction of PD symptoms by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment.<sup>[95,96]</sup> Furthermore, TLR4 and  $\alpha$ -synuclein are both necessary for LPS-induced neurodegeneration in mice.<sup>[97,98]</sup> Therefore, mouse models support the importance of TLR4 in PD, but make no suggestion













