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Evaluating the role of Toll-like Receptors in diseases of the Central Nervous system

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Short title

Toll-like receptors and CNS diseases

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Abstract

A key part of the innate immune system is a network of pattern recognition receptors (PRRs) and their associated intracellular signalling pathways. Toll-like receptors (TLRs) are one such group of PRRs that detect pathogen associated molecular patterns (PAMPs). Activation of the TLRs with their respective agonists results in the activation of intracellular signalling pathways leading to the expression of proinflammatory mediators and anti-microbial effector molecules. Activation of the innate immune system through TLRs also triggers the adaptive immune response, resulting in a comprehensive immune program to eradicate invading pathogens. It is now known that immune surveillance and inflammatory responses occur in the central nervous system (CNS). Furthermore it is becoming increasingly clear that TLRs have a role in such CNS responses and are also implicated in the pathogenesis of a number of conditions in the CNS, such as Alzheimer's, stroke and multiple sclerosis. This is likely due to the generation of endogenous TLR agonists in these conditions which amplifies a detrimental neurotoxic inflammatory response. However TLRs in some situations can be neuroprotective, if triggered in a favourable context. This review aims to examine the recent literature on TLRs in the CNS thus demonstrating their importance in a range of infectious and non-infectious diseases of the brain.

1. Introduction

The survival of an organism critically depends on its ability to defend itself from invading pathogens. In higher organisms this is provided by the existence of an innate and adaptive immune system. The innate immune system is composed of a series of germ line encoded pattern recognition receptors (PRRs) that can detect conserved pathogen associated molecular patterns (PAMPs) expressed on microorganisms. The adaptive immune systems responds to microbial infection by expressing antigen receptors through somatic recombination and is characterised by the existence of immunological memory. Activation of the innate immune system leads to the production of inflammatory mediators, antimicrobial effectors and orchestration of the adaptive immune response leading ultimately to pathogen elimination. Several important PRRs of the innate immune system include Toll-like receptors (TLRs), Rig like Receptors (RLRs), Nod-like Receptors (NLRs) [1] and the recently identified Aim2 like receptors (ALRs) [2]. Although these PRRs, which have similarities and differences in terms of protein domain structure and subcellular localisation, they act together to defend against invading pathogens (figure 1).
For example TLRs consist of leucine rich repeats (LRRs) and a Toll-Interleukin 1 receptor (TIR) domain and are located on the cell surface and endosomes. RLRs contain a helicase and CARD domains and are located in the cytoplasm. The cytoplasmic NLRs consist of NACHT, PYRIN, LRRs and CARD domains. Finally ALRs contain a PYRIN domain and HIN domains and are also located in the cytoplasm (figure1).

The family of TLRs was initially discovered in Drosophila, where *Toll*, was shown to have a role in dorsal-ventral polarity. It was then discovered that *Toll* showed similarity to the human

interleukin-1 receptor. Later it was shown that Drosophila *Toll* did have a role in immunity, in particular against fungal infection and this led to the search for human Toll-like receptors, the first of which was discovered in 1997 [3]. It is now known that there are 10 functional TLRs in humans and 12 in mice. TLR1-9 are conserved in both human and mice, mouse TLR10 is non functional, while TLR11, 12 and 13 are not present in the human genome [4].

TLRs detect a wide range of PAMPs that are found on bacteria, viruses, fungi and parasites which include proteins, lipids, lipoproteins and nucleic acids. TLRs are expressed on a variety of immune and non immune cells such as monocytes, dendritic cells (DCs), epithelial cells and fibroblasts. TLRs are found either on the cell surface or in intracellular compartments in endosomes. These receptors function as hetero or homodimers, for example TLR1/2 and TLR2/6 heterodimers are found on the cell surface and detect triacyl and diacyl lipoproteins respectively (figure 2). TLR4 and TLR5 are also found on the cell surface and detect lipopolysaccharide and flagellin respectively (figure 2). By contrast the endosomal TLRs detect nucleic acids. TLR3 detects double strand RNA (dsRNA) and its synthetic analog, polyinosine-deoxycytidylic acid (polyI:C), TLR7 and 8 detects viral single stranded RNA while TLR9 detects unmethylated CpG dinucleotides of microbial origin [4] (figure 3).

1.1 TLR signalling

TLRs are type 1 membrane spanning receptors that consist of extracellular LRRs, a transmembrane domain and a cytoplasmic TIR domain, the presence of which defines membership of the family. Downstream signalling is achieved by the presence of cytoplasmic TIR containing adaptor proteins. Five of these proteins exist MyD88, Mal, TRIF, TRAM and

SARM. MyD88 is the prototypical member of the group and is required to transmit all TLR signals with the exception of TLR3 which uses TRIF only. Both TLR2 and TLR4 require Mal as a bridging adapter to MyD88. In contrast TLR4 signalling following LPS stimulation requires Mal, MyD88 and TRAM as a bridging adapter to TRIF to mediate MyD88 independent signalling [5]. All TIR adaptors have important positive roles in mediating intracellular signalling following TLR stimulation except SARM, which has been reported to inhibit TRIF dependent TLR signalling [6].

Upon TLR stimulation the respective adaptors are recruited to the cytoplasmic tails of the receptors. This leads to the recruitment of IL-1 receptor–associated kinases (IRAKs). IRAK4 recruitment to MyD88 leads to the phosphorylation of IRAK1 and an association with TRAF6. TRAF6 is an ubiquitin E3 ligases and with its E2 counterparts Uev1A and Ubc13 triggers the ubiquitination of both TRAF6 itself and other substrates. This non degradative K63 linked ubiquitinated TRAF6 induces the recruitment of TAB2 and TAB3 via specific ubiquitin-binding domains. The degradation of IRAK1 allows this TRAF6 complex to move into the cytosol. In addition to IRAK1, IRAK2 has an important role in this process as it has been shown to be required for TRAF6 ubiquitination and NFκB activation [7]. Through TAB2/3, TAK1 is recruited to the TRAF6 complex and activated. This in turn allows TAK1 to stimulate the activity of the kinases complex of IKKα and β, through the regulatory subunit NEMO. This results in the phosphorylation and degradation of the NFκB inhibitor IκBα. This permits the translocation of NFκB into the nucleus to activate the expression of pro-inflammatory cytokines. In addition TAK1 can activate the p38 and JNK MAP kinase pathways through the phosphorylation of MKK3/6 and MKK4 respectively. Alternatively TLR activation of the ERK MAP kinase pathway is dependent upon Tpl2 acting through MKK1/2 [8]. A separate pathway to NFκB and

MAP kinase activation following TLR3 and TLR4 stimulation is mediated by TRIF. This adaptor initiates a signalling cascade through RIP1 binding, and through its adaptor TRADD, triggers K63 linked ubiquitination of RIP1, which is independent of TRAF6. This allows the recruitment of TAB2 and subsequent activation of TAK1, thus allowing IKK and MAP kinase activation. In addition RIP1 can also activate NFκB through FADD and caspase 8. Stimulation of TLRs leads to the production of type 1 interferons (IFNα/β). Production of these mediators following TLR3 and TLR4 stimulation relies on the MyD88 independent TRIF pathway. Following TLR3 and TLR4 stimulation TRIF recruits TRAF3. This results in K63 linked polyubiquination of TRAF3 and the recruitment of TANK, NAP1and SINTBAD. This allows the movement of TBK1 and IKKε to the receptor complex. This complex activates TBK1 and IKKε which are the kinases for IRF3 and IRF7. Phosphorylation of these transcription factors lead to their dimerisation and translocation into the nucleus resulting in the expression of IFNα/ β (for a recent review see [9]). It is important to note that all type 1 interferon production following TLR stimulation, including that followed by TLR4 stimulation, is initiated at the endosome (Figure 2 and 3).

1.2 TLR expression in cells of the brain

TLRs expression has been found in many cell types of the brain in both mice and humans and a number of substantial differences in TLR expression have emerged between these species. For example human astrocytes only express TLR3 mRNA [10] whereas murine astrocytes express TLR2, 4, 5 and 9 mRNA [11]. Murine primary cortical neurons express TLR2, 3 and 4 at the mRNA and protein level [12]. TLR2 and TLR6 protein was also shown to be expressed in primary brain neurons under control conditions, while protein levels for TLR4, 7 and 8 was induced by parasitic infection [13]. In contrast human neurons only express TLR3 [14], while

both mouse and human microglia express mRNA for TLR1-9 [15] [16] (Table 1). The expression of TLR3 is particularly interesting as it is highly expressed in both murine and human astrocytes and highly expressed in the resting CNS [17] suggesting that it may have vital immune or homeostatic roles in the brain.

2 Inflammation in the brain

While it was once thought that the brain and CNS were immune privileged it is now known that immune surveillance and inflammatory responses occur in the brain [18] [19]. Acute inflammation in the brain is protective, since it removes pathogens, cellular debris and leads to tissue repair. However prolonged inflammation in the brain causes progressive neurotoxicity leading to irreversible neuronal loss observed in neurodegenerative disease [20]. This is due to the exquisite sensitivity of neurons to inflammatory molecules such as reactive oxygen species (ROS) and the limited ability of the brain to regenerate neurons [19]. While neurogenesis occurs primarily in development, the growth of new neurons occurs throughout adult life. The inflammatory mediators released during mild acute inflammation promote neurogenesis, while the inflammatory mediators released during prolonged inflammation inhibits neurogenesis [21]. New neurons are generated from neural progenitor cells (NPCs) and this is affected by the inflammatory process. Not alone do neurons need to be newly generated but these neurons must differentiate, migrate, survive and integrate correctly into the CNS circuitry in order to be functionally relevant and therefore constitute successful neurogenesis [21]. For example although in Alzheimer's disease (AD) there is an increase in neurogenesis, these neurons do not mature and survive [21]. Therefore the brain is sensitive to prolonged inflammation and thus this process must be tightly regulated.

TLRs are expressed and elicit functional signalling pathways in the CNS [18], and it has been suggested that TLRs might be the initial trigger for inflammation in the CNS. The observation that TLR expression is increased in the brains of patients with AD and the brain and spinal cords of multiple sclerosis (MS) patients supports this notion and also suggests that TLRs may be involved in the progression of these diseases [18]. It is noteworthy that controlled inflammation plays a role in protecting neurons in the CNS [18], hence in some situations TLRs might confer a neuroprotective immune response. In addition there is evidence to suggest that TLRs expressed in the brain have functions distinct from immunity, for example, TLRs are functional in adult neurogenesis [18] and TLR 8 expression on neurons triggers apoptosis suggesting a role in CNS homeostasis [18]. With regard to infectious diseases, TLR9 activation may be involved in the induction of meningitis and TLR3 expression is enhanced during infection with rabies or herpes simplex virus [18]. These observations all indicate that TLRs are important in infectious and non infectious diseases of the brain. This review aims to provide a brief summary of the role that TLRs play in some of these illnesses.

2.1 Tissue damage releases endogenous TLR ligands

It is becoming increasingly clear that TLRs are activated by endogenous ligands generated in situations of sterile inflammation. Examples of these ligands include heat shock proteins (HSPs), fibrinogen, fibronectin, soluble hyaluronan, oxidized LDL, mRNA, gangliosides, fatty acids and high mobility group box 1 protein (HMGB1) [18] [19]. These danger associates molecular patterns (DAMPs) are release from dying cells and stimulate TLRs on microglia to produce

neurotoxic inflammatory mediators [19]. This model is supported by the observation that HSP60, released from dying cells of the CNS and stimulates TLR4 on microglia to trigger the production of nitric oxide (NO) which is toxic to neurons [22]. Therefore by the release of these "alarmins", this intrinsic activation of TLRs serves to amplify the inflammatory response in the absence of infection and contribute to inflammatory diseases of the brain.

2.2 Role of Microglia in inflammation

Microglia are a vital part of immunity in the CNS as they can activate both innate and adaptive immune responses. Microglia express many TLRs and are the resident macrophages of the CNS. Under normal conditions in the CNS these cells are inactive and express low levels of MHC and co-stimulatory molecules. These inactive microglia are important in immune surveillance as they constantly monitor the CNS microenvironment through pinocytosis. Infection or injury in the CNS results in the activation of microglia leading to their increased proliferation, motility, phagocytosis and release of cytokines and ROS [23]. Activation of microglia also results in increased expression of MHC and co-stimulatory molecules and stimulates CD4 and CD8 T cell responses, and therefore serve as important APCs of the CNS [23]. Microglia are of myelomonocytic origin and in addition to TLRs, these cells express CD11b and CD45 [23]. It has been noted that microglia are activated in all diseases of the CNS and they are among the first cells found at the site of tissue injury and infection, and function to recruit other immune cells [19]. It has also been found that activation of TLRs on microglia can result in injury to neurons and oligodendrocytes [19].

3. TLRs in Alzheimer's disease

AD is the most common neurodegenerative disease which is characterised by progressive neuronal death and memory loss. Although the etiology of the disease is unknown, accumulation of β amyloid (Aβ), leads to the development of extracellular senile plaques and intracellular neurofibrillary tangles. Aβ is a 42 amino acid ($A\beta_{42}$) fragment derived from the amyloid precursor protein [24] [25] and it is the inflammatory response to this pathological agent that is central to the disease [26]. Microglia are found in an actived state around senile plaques in the brains of AD patients and are considered to be important in the pathogenesis of the disease. Activation of microglia results in the production of NO, oxygen free radicals, proteases, adhesion molecules and proinflammatory cytokines such as TNFα, IL-1β, LT-α, and IL-6 [27-31]. It is thought that the overproduction of these inflammatory mediators is important in the degenerative process in patients with AD [32].

3.1 Role for TLRs in Alzheimer's disease

There is a growing body of evidence that suggests that TLRs are important in AD, in particular those TLRs that are expressed in microglia. For example animal models of AD and patients with AD exhibit increased expression of CD14, (a co-receptor for TLR4), TLR4 and TLR2 [33-36], which are thought to occur independently in response to the presence of Aβ. Interestingly a polymorphism in TLR4 Asp299Gly resulted in a 2.7 fold reduction in risk for late onset AD [37]. Senile plaque associated microglia show increased mRNA levels of TLR 2, 4, 5, 7 and 9 [38] and

a physical interaction between CD14 and fibrillar Aβ (FAβ) was demonstated by Reed Geaghan et al [39]. The signal transduction cascades triggered by FAβ is identical to those triggered by TLR agonists [39] and Aβ induction of NFκB dependent genes requires TLR2, TLR4 and CD-14 [39], thus indicating that TLRs are important in sensing and responding to the presence of Aβ.

Studies in primary mouse microglia and BV-2 microglia cells showed that TLR2 mediates activation of microglia in response to FAβ, leading to the expression of proinflammatory cytokines, inducible NO synthase and integrin markers. This response was dependent upon MyD88 and microglia from mice lacking TLR2 were not activated by FAβ [32]. Furthermore in a functional screen using antisense knockdown, while knockdown of TLR2 reduced TNF release in microglia stimulated with FAβ, knockdown of TLR 4, 6, 7 and 9 had no such effect [32].

A role for CD14 in AD was demonstrated as CD14 associates with Aβ and is involved in the phagocytosis of Aβ. Immunohistochemical staining of brains of AD patients showed strong expression of CD14 in senile plaques and parenchymal microglia which was not observed in age matched controls [34].

Further evidence suggesting a role for TLRs in microglial responses to Aβ was provided in a study where microglia derived from TLR2, 4 and CD14 KO mice were defective in Src-Vav-Rac and p38 MAPK signalling and showed reduced ROS production and phagocytosis in response to FAβ [39]. This suggests that TLR2, TLR4 and CD-14 are part of the receptor complex that responds to FAβ. This cellular complex also consists of cell surface receptors such as CD36, $\alpha_6\beta_1$

integrin, CD47 and scavenger receptor A, which, along with TLRs 2, 4 and CD14 can bind to FAβ to trigger multiple intracellular signaling cascades [39-41].

The G protein-coupled formyl peptide receptor-like 1 (FPRL1) and its mouse homologue mFPR2 is a receptor and mediates the internalisation of Aβ peptide, suggesting that this receptor may be important in the disease progression of AD. In addition FPRL1 mediates the chemoatractic activity of microglia in response to Aβ peptide which may lead to the recruitment of microglia to AD lesions. Interestingly stimulation of both TLR2 and TLR4 lead to the upregulation of mFPR2 and enhances the uptake of Aβ peptide and increases the chemotactic functions of microglia [42, 43]. The authors of these studies propose that activation of TLR2 and TLR4 on microglia may promote microglial responses in the CNS especially in pathological situations such as AD where the Aβ agonists for mFPR2 are elevated, and thereby amplify the inflammatory response in AD [43]. This intrinsic TLR activation may be mediated by a range of host derived molecules which may activate TLR2 and TLR4, such as HSP60, HSP70, GP96 and HMGB1, which are all increased in inflammatory conditions [43].

Though most studies of TLRs in AD have focused on microglia, TLRs may be important in the disease progression of AD that are expressed on cells of the CNS other than microglia. For example TLR4 was shown to mediate neuronal apoptosis in response to $A\beta_{42}$ [44], indicating a complex relationship between the various cells expressing TLRs in the presence of Aβ. In AD it is proposed that CD14 is a double edged sword. While the role of CD14 in the phagocytosis of Aβ is thought to be beneficial, CD14 mediated cellular activation and release of neurotoxic

products is thought to be detrimental [34]. The authors propose that in early disease that low concentrations of Aβ might trigger CD14 dependent phagocytosis of Aβ peptide, while in more advanced AD, higher concentrations of Aβ may lead to CD14 dependent cellular activation and release of neurotoxic mediators [34].

3.2 Unanswered Questions in Alzheimer's disease

It is currently unknown why microglia in AD fail to phagocytose and clear Aβ deposits in the brain since microglia *in vitro* are proficient in this function [41]. It is thought that phagocytosis of Aβ peptide by microglia is a mechanism of host defence, however long term exposure of microglia to Aβ peptide may result in fibrillary deposition [43]. It has been proposed that clearance or deposition of Aβ peptide may be determined by factors such as Aβ peptide burden and the duration of cell exposure [43]. Interestingly it has been reported that proinflammatory cytokines reduces the function of the phagocytic machinery of microglia, which can be relieved by anti-inflammatory cytokines, suggesting a possible strategy for the treatment of AD [45]. However it remains unknown if TLR induction of phagocytosis is impaired in AD [41], thus raising the important question of whether inhibitors or modulators of TLRs or their signaling pathway are produced in the brains of AD patients. A number of studies have reported that TLR activation is associated with increased clearance of Aβ, which combined with some indications that TLRs are somehow dysfunctional in AD, raises the prospect that the use of TLR agonists may be beneficial in AD [46]. Thus in the future it may be possible to develop therapeutics that enhance TLR dependent microglial phagocytosis of Aβ and yet limit the release of neurotoxic proinflammatory cytokines. In this regard an interesting recent study has shown that the TLR9

agonist CpG increased phagocytosis of Aβ without the accompanying release of the neurotoxic NO or glutamate [47].

4. TLRs in Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory demyelinating disease and while the exact cause is unknown, an environmental or infectious based etiology has been proposed [48]. The pathogenesis of the disease is based on cell mediated immunity. The disease may occur in a relapsing-remitting form or in a progressive form where disease severity worsens over time [48]. The disease pathogenesis occurs in two main stages, the initial priming/activation phase in which self reactive lymphocytes are activated and a later effector phase where these cells invade the CNS and cause tissue destruction. DCs play a vital part of the initial T cell priming phase and maintenance of the disease process. Activation of these DCs occurs due the presence of endogenous danger signals which leads to the breakdown of tolerance and the development of an autoimmune response [49].

In order to study MS, mouse models are employed where experimental autoimmune encephalitis (EAE) is triggered by the injection of myelin proteins together with complete freunds adjuvant (CFA) containing *Mycobacterium tuberculosis*, which lead to the development of myelin specific CD4+T cells [50]. In EAE Th17 cells are particularly important for the induction of the disease [48] and these cells enter the CNS after Th1 cells[51]. However the importance of Th17 cells in mouse models of autoimmune neuroinflammation is controversial [52].

A number of studies have implicated TLR ligands in the development of EAE [53]. In support of this, MyD88 knockout mice are completely resistant this disease, since they fail to develop Th1 and Th17, which are essential in the pathogenesis of EAE [54]. However while a role for TLR signalling in the development of myelin specific Th17 cells has been established the stimulatory TLRs for these Th17 cells have not yet been identified [54].

While TLR signalling is required in EAE, the role of individual TLRs in the condition is more complex. For example TLR4 knockout mice have greater disease severity which is associated with increased Th17 function [54], while determining the contribution of TLR9 has produced conflicting results [49, 54]. In contrast TLR3 stimulation is protective in EAE due to the induction of IFNβ [55] which has led to the suggestion that TLRs inducing interferon is protective, whereas TLR stimulation that leads to proinflammatory cytokine expression adds to disease severity [56]. In addition to this, the immune cells which express TLRs have an important bearing on the disease. In mice whose B cells are lacking MyD88 or TLR2 and TLR4, chronic EAE develops, whereas those animals with TLR9 deficient B cells, recovered at a similar rate to wild type (WT) mice [48, 57] (Table 2). This might be due to TLR dependent induction of IL-10 from B cells which prevents the release of IL-6 from DCs [57]. These data support a model where TLR signalling in B cells may limit T cell mediated autoimmunity in EAE [48].

TLR signalling has been found to be necessary for the development of EAE triggered by the injection of myelin proteins, however TLR agonists are present in the adjuvant preparations, therefore it is difficult to extrapolate how data from these studies relate to the role of endogenous TLR ligands, TLR receptors and TLR signalling in human multiple sclerosis. Since it is known

that TLR3 and TLR4 are upregulated in human MS [58], further work is required to establish the significance of the TLR network in the human disease.

5. TLRs in Stroke

Stroke results from a loss in blood supply to a specific region of the brain that leads to ischaemic injury (Bejot et al 2009). This causes metabolic stress and death in neurons resulting from lack of ATP. This neuronal death is initially caused by necrosis and following ischaemia and reperfusion, cell death occurs by apopotosis [59]. Stroke is a condition of complex pathology and the outcome to stroke depends on multiple cell types and inflammatory mediators and these factors can be detrimental or beneficial depending on the duration magnitude and timing of response [59].

Ischaemia induced cells death triggers the activation of microglia, production of proinflammatory cytokines such as TNFα, IL-1β and chemokines CCL2 and CCL3.This leads to the infiltration of inflammatory cells such as leukocytes, macrophages and neutrophils from the periphery. These cells release neurotoxic or neuroprotective mediators that affect the outcome to stroke. For example infiltrating macrophages in stroke release TNF and IL-1β, and while it was shown that IL-1β is neurotoxic and inhibitors of IL-1β are beneficial, determining whether TNFα has a positive or negative effects is challenging and has produced conflicting results [59].

There is evidence to suggest that the recruitment of inflammatory cells to the injured site is detrimental in the outcome to stroke [60]. Furthermore it was also shown that inhibition of IL-8, NFκB, and decreased leukocyte infiltration all improve stroke outcome [59].

As stated previously not only can TLRs sense infection but they can also sense tissue damage. Microglia activation in stroke may be dependent on TLRs to trigger the release of a range of inflammatory mediators to attract other immune cells to the site of injury [59]. Myeloid DCs are recruited to the infarct site and mediate the recruitment of T cells by the release of IFN-<gamma>. The presence of T cells at the infarct site is both beneficial and detrimental. Th1 cells release the proinflammatory cytokines IL-2, TNFα and IFN-<gamma>, while Th2 cells produce the anti-inflammatory cytokines IL-10 and IL-13. In addition Tregs produce the neuroprotective IL-10, which is dependent upon TLR signalling [59].

5.1 Detrimental role for TLRs

During ischaemic injury the blood brain barrier (BBB) is disrupted due to the release of proteases such as matrix metalloproteinases (MMPs). This results in the release of endogenous TLR ligands into the periphery which may activate immune cells of the periphery bearing TLRs. Therefore TLRs signalling activated after stroke occurs both in the periphery and the CNS and has been suggested to be a bridge between both locations [59].

A variety of studies using mouse models for stroke suggest that the release of endogenous ligands activate TLRs to contribute to the tissue injury caused in this condition. Many of these studies have implicated important roles for TLR2 and TLR4 in this pathological process.

Following cerebral ischaemia TLR4 is activated by HMGB1 in neurons and astrocytes to trigger the release of MMP9 [61]. In addition the authors also show infarct size was decreased in mice with a missense mutation in TLR4 [61]. Consistent with this, mice lacking TLR4 but not those lacking TLR3 or TLR9 were shown to have reduced infarct area following ischaemic challenge [62, 63] (Table 2). In addition there was a reduction in the number of microglial cells at the infarct site in mice lacking TLR4.

The expression of TLR2 is upregulated in cerebral ischaemia and similar to TLR4, mice lacking TLR2 had smaller infarct size compared to WT mice [64]. The scavenger receptor CD36 is a co-receptor for TLR1/2 and TLR2/6 heterodimers and cerebral injection of ligands for TLR1/2, but not ligands for TLR2/6 or TLR4, produced an inflammatory response that was dependent on CD36. This indicates that CD36 is necessary for TLR1/2 signalling in the brain [65] (Table 2).

Further support for a detrimental role of TLRs after stroke was provided in a study using animal models of glucose and energy deprivation. The authors of this study showed that inhibition of TLR2 and TLR4 on neurons prevented JNK activation and neuronal death after energy deprivation [12].

In the ischaemic brain the activation of NFκB but not IRF3 is increased, and this increase in NFκB activation was shown to be independent of TRIF [63]. In addition neurological defects and infarct size was not altered in mice lacking TRIF compared with controls when subjected to ischaemic challenge. Furthermore an in vitro model of stroke where oxygen and glucose deprivation is used, it was found that activation of the TRIF pathway by poly(I:C) reduced this

mode of neuronal death [66]. Thus it appears that MyD88 dependent signalling after stroke is detrimental while TRIF mediated signalling might be beneficial and therefore having important implications for neuroprotection.

5.2 TLRs in neuroprotection

It has been established that TLR activation after ischaemia by endogenous ligands contributes to tissue damage in stroke. However TLR activation before ischaemia was shown to be protective [66]. Systemic administration of LPS by intraperitoneal injection prior to ischaemic challenge induced a neuroprotective reponse. The protective effect of LPS preconditioning is dependent on TRIF and IRF3 and this neuroprotection is likely due to the production of type I interferons, as direct intracerebroventricular administration of IFNβ at the onset of stroke is neuroprotective [66]. In addition it was shown that Pam3CSK4 administration 24 hrs before cerebral ischaemia reduced infarct size [63] and pretreatment of TLR9 agonists CpG before stroke also conferred neuroprotection, at least partly due to the release of TNF alpha [67]. TLRs are also involved in ischaemic preconditioning where ischaemia induced of a short duration provides resistance to subsequent challenge thus conferring ischaemic tolerance [68]. This protective effect was shown to be dependent upon TLR4 to upregulate TNFα, inducible NO synthase, cyclooxygenase 2 and NFκB [68].

6. TLRs and Glioma

Malignant glioma is the most common and most aggressive form of primary brain tumour in adults which have a poor prognosis [69]. In these tumours there are high levels of infiltrating microglia, yet in the immunosuppressive tumour microenvironment, these microglia are unable to induce an effective anti-tumour T cell response [23]. The immunosuppressive properties of glioma include, high levels of infiltrating Tregs and secretion of immunosuppressive TGFβ and IL-10 [70, 71]. Other function of microglia that are inhibited by glioma include, phagocytosis, antigen presentation and secretion of proinflammatory cytokines [23]. An effective immunotherapy has been proposed to be one in which alleviates the immunosuppressive micro-environment of glioma and yet triggers an effective anti-tumour immune response [71].

A number of studies in mouse models of glioma have showed that direct intracranial injection of CpG elicits potent anti-tumour effects [72]. These TLR9 agonists are powerful immune stimulators that induce cytokine secretion which results in the activation of NK and T cell responses [72]. In one such study a single intratumoral injection of CpG inhibited glioma growth and cured 80% of mice with glioma [71]. The use of TLR9 KO mice showed that the effectivness of CpG was due to the expression of TLR9 on non tumour cells. In addition treatment with CpG increased tumour infiltrating CD4+ and CD8+ effector T cells and increased the ratio of CD4+ effector T cells to regulatory T cells [71]. Interestingly, surviving mice treated with CpG and re-challenged with glioma rejected the tumour which shows that CpG conferred a protective immune memory [71]. In a similar study, multiple low doses intratumoral injection of CpG eliminated gliomas in 70% of mice by inducing the effector functions of NK cells [73]. Importantly the anti-tumour effect of CpG was shown to be dependent upon microglia [23].

The anti-glioma effect of TLR stimulation might not be limited to TLR9, as intratumoral injection of Pam3Cys-SK4 or R848 to stimulate TLR1/2 and TLR7 respectively also showed survival benefit. In contrast TLR3 stimulation with poly(I:C) or LPS stimulation of TLR4 failed to show anti-tumour effects [71].

In addition to microglia, DC function may also be augmented to surmount the immunosuppressive environment of glioma and enhance the anti-tumour immune response. DCs of the brain are located at the choroid plexus, the meninges and the perivascular spaces. These cells are not normally found in the brain parenchyma but can are found at brain lesions [74]. TGF-β2 is a potent immunosuppressive cytokine that is produced by gliomas to inhibit T cell function. *In vitro* stimulation of human DCs by a maturation cocktail containing the TLR ligands Poly(I:C) or R848 with TNFα, IL-1β and IFN induced MHC II expression and IL-12 secretion, that was unaffected by TGF-β2 [74]. This suggests that TLR stimulation of DCs may trigger an effective immune response in the immunosuppressive environment of glioma [74].

In addition to studies in mice a number of human studies have been undertaken to determine the relationship between TLR expressing microglia and glioma. Microglia from surgically resected glioma showed TLR expression patterns similar to that of microglia from healthy individuals. However compared with microglia from healthy individuals, glioma associated microglia exhibited reduced tumour cytotoxicity, thus indicating that glioma impairs the function of microglia [70]. In other human studies TLR9 mRNA expression levels have been evaluated in glioma where it was found that in 37 patients TLR9 mRNA was expressed at different levels, suggesting that evaluating TLR9 levels may identify patients more likely to better respond to CpG treatment [72].

Promising results from mouse models of glioma treated with CpG-ODN have lead to the use of CpG in clinical trials. A phase 1 clinical trial was undertaken using intratumoral administration of CpG-28 in recurrent glioblastoma, which showed some response without adverse side effects. [75]. In a follow-up phase II study, again using CpG-28 in recurrent glioblastoma, some response in progression-free survival at 6 months was observed [76] (Table 3).

So far we have evaluated ways in which TLR expressing microglia might be manipulated therapeutically to trigger an anti-tumour immune responses, however some evidence actually points to microglia mediating immunosuppression in glioma and possibly contributing to tumour proliferation and progression [23]. It is thought that microglia through the production of MMPs, VEGF and EGF may contribute to tumour migration, angiogenesis and proliferation respectively [23]. It is also possible that endogenous TLR ligands produced from dying tumour cells such as heat shock proteins and HMGB act on TLRs expressed on microglia to contribute to tumour proliferation. Indeed it is established that TLRs can be both beneficial and detrimental in cancers generally having both anti-tumour and pro-tumour effects [77].

7. Role of TLRs in Chronic pain

It was once thought that pain was mediated solely by neurons, however it is now becoming clear that glial in the spinal cord contributes to the initiation and maintenance of pathological pain from a variety of sources [78, 79]. Spinal cord glia are activated by sensory signals from the periphery, and similar to infection, release proinflammatory cytokines that contribute to pain [78, 80]. Neuropathic pain in a chronic sensory disorder triggered by damage to sensory peripheral or

central nerves and leads to spontaneous pain and enhanced pain responses to both noxious and innocuous stimuli [81]. This type of pain in not protective and is itself regarded as a pathological condition [82]. In contrast to inflammatory pain, treatments for neuropathic pain is lacking, and therefore is a requirement to understand its molecular pathogenesis [83]. It is now thought that the basis of neuropathic pain requires immune activation in the CNS, where the production of chemokines and cytokines triggers the expression of pain mediators such as glutamate and NO [80, 84-86]. Critically proinflammtory cytokines such as TNF, IL-1 and IL-6 have not been associated with the generation of normal non pathological pain, yet these mediators are all associated with conditions where pain is exadurated [78]. Furthermore stimulation of perispinal microglia by bacterial cell walls or viral envelope proteins induces hyperalgesia (increased sensitivity to pain) [78].

Animal models of neuropathic pain involve the use of L5 spinal nerve ligation and monitoring behavioural sensitivity, a hallmark of neuropathic pain [86]. The use of animal models of neuropathic pain reveal activation of microglia and astrocytes in the spinal cord and the production of IL-1β and TNFα which are important in the initiation and maintenance of neuropathic pain [82].

Accumulating evidence indicates that TLRs have an important contribution to the development of neuropathic pain. It is now though that the release of endogenous DAMPs following nerve damage leads to the activation of astrocytes and microglia via TLRs to increase the expression of proinflammatory cytokines and chemokines which induces the production of pain mediators [86].

It has been demonstrated using mouse and rat models of neuropathic pain that lack of TLR4 function reduces nerve injury induced pain sensitivity [87]. Using knockout mice it was shown that CD14 contributes to TLR4 dependent nerve injury mediated neuropathic pain but not in the nociception of physiological pain [86]. It was also shown that this role of CD14 was mediated by TLR4 dependent pathways. However the endogenous ligand that triggers the CD14-TLR4 signalling pathway following nerve injury was not identified, but may include one or more of HSPs, HMGB1, and β defensins [86]. The same study also showed that spinal cord microglia become reactive following nerve injury induced neuropathic pain.

Intriguingly it has been demonstrated that opioids activate microglia via TLR4 and morphine induces TLR4 signalling in HEK 293 cells [82]. Opioid activation of microglia via TLR4 produces undesirable effects such as a reduction of opioid analgesia and increases opioid tolerance, dependence, reward and respiratory depression [88]. Since the desired beneficial effects of opioids are mediated by opioid receptors on neurons, Watkins and co-workers propose that the desired effects of opioids may be separated from the unwanted side effects of TLR4 activation on glia by the use of TLR4 inhibitors. Furthermore the point is also made that the use of such a strategy may be a useful treatment of neuropathic pain [88].

Antisense knockdown of TLR3 reduced the activation of spinal microglia and inhibited the increase of spinal proinflammatory cytokines IL-1β, IL-6 and TNFα following nerve injury. Interestingly intrathecal administration of the TLR3 agonist poly(I:C) produced many of the features characteristic of nerve injury [83]. In addition TLR3 knockout mice failed to develop tactile allodynia (painful response to non painful stimuli) after nerve injury or poly(I:C) injection.

These data indicate that TLR3 is critical for the development of neuropathic pain [83]. Since TLR3 binds mRNA released from necrotic cells it has been suggested that this may be the mechanism responsible for the TLR3 role in neuropathic pain [79]. This indicates that blockade of TLR3 in spinal cord glial cells may potentially be beneficial in the treatment of neuropathic pain [83].

Using knockout mice it was shown that TLR2 also contributes to nerve injury induced pain hypersensitivity by activating microglia and astrocytes and inducing proinflammatory gene expression [79, 81]. It has been proposed that damage to nerve axons may trigger this TLR2 dependent activation of microglia [79].

All of these studies suggest that TLRs are important in the molecular pathogenesis of neuropathic pain and raises the possibility that blockade of TLRs on glia may provide therapeutic benefit in the treatment of this devastating condition.

8. Role of TLRs in infectious diseases of the brain

The location of TLR-expressing cells in the brain is indicative that these PRRs are likely to be important in sensing invading pathogens into this organ. For example microglia expressing TLRs are located at regions exposed to the circulation such as the circumventricular organs, meninges and choroid plexus [17, 18]. TLR expression in the CNS is enhanced by bacterial and viral infection in order to initiate an inflammatory response [17]. Finally stimulation of TLRs on

microglia and astrocytes with either TLR ligands or pathogens results in the production of a variety of inflammatory mediators [17].

8.1 TLRs and Cerebral malaria

It has been established that TLRs recognise malaria parasites and their metabolites and studies have been undertaken to understand the role of TLRs in malaria infection. In particular attempts have been made to address the role of TLRs in the development of cerebral malaria (CM), which is a lethal complication of malaria infection in humans [89]. This condition is characterised by reduced consciousness or coma and generalized convulsions [90]. In one study it has been demonstrated that the pathogenesis of CM is mediated by MyD88 dependent TLR signalling. Compared with WT and TRIF knockout (KO) mice, survival but not parasitemia was increased in the TLR2, TLR9 and MyD88 knockout mice following plasmodium berghei ANKA (PbA) infection. This suggests that this TLR axis does not confer protective immunity to PAb infection but rather contributes to the pathogenesis of infection [91]. In contrast TLR 4, 5 and 7 showed no involvement in response to infection with PbA, as mice lacking these TLRs exhibited no survival benefit compared with WT mice. While systemic parasitemia was comparable in the WT, TRIF and MyD88 knockouts, parasite sequestration and hemozoin load in the blood vessels of the brain were lower in the MyD88 KO mice [91]. Furthermore a number of pathological features in the brain associated with CM were dependent upon MyD88, such as the infiltration of CD8+, CCR5+ T cells, CD11c+ dendritic cells and the increased expression of inflammatory response genes Granzyme B, Lipocalin 2, Ccl3 and Ccr5 [91]. Further evidence in support of a role of TLR2 and

TLR9 involvement in malaria comes from the observation that Glycosylphosphatidylinositol (GPI) and hemozoin have been reported to be ligands for TLR2 and TLR9 respectively [91].

In contrast a study using mice lacking TLR1, 2, 3, 4, 6, 7, 9, CD14, MyD88, Mal or TRIF, showed that these mice exhibited similar sensitivity to lethal CM development following PbA infection compared to WT mice. In addition vascular permeability of the brain did not differ between WT and MyD88 deficient mice with CM [89]. This led to the authors to conclude that the development of CM following PbA infection is independent of TLRs and their signalling adaptors.

A further study again using PbA infection as a model of CM examined the role of TLRs. Using triple TLR2/4/9-deficient mice it was demonstrated that the development of CM was not affected by the absence of these TLRs [90]. In these mice the induction of ICAM1 on brain endothelium and recruitment of T cells to the brain were unaffected. This is in agreement with the study of Togbe et al [89]. The reason for these conflicting observations is unknown and confusing since these studies all used similar models of CM. Therefore the significance if any, of the detection of malaria products by TLR2 and TLR9 to the development of CM is currently unknown. However despite the conflicting data regarding TLRs in murine models of CM, a polymorphisms in TLR9 has been associated with altered levels of IFNγ in children with CM [92].

8.2 TLRs and Herpes Simplex Encephalitis

Studies in mice have shown that microglia respond to herpes simplex virus-1 (HSV-1) infection by the TLR2 dependent production of a range of cytokines and chemokines. Since mice lacking

TLR2 exhibit reduced mortality and neuroinflammation from brain infection by HSV-1, it is suggested that TLR2 can mediate the pathogenesis of this viral infection [93, 94]. It has also been found that TLR2 mediates apoptosis in microglia in response to HSV infection [95]. A recent *in vitro* study has shown that microglia respond to HSV-1 infection by producing ROS leading to neurotoxicity, an effect which was reduced in microglia from mice lacking TLR2 [96]. It appears therefore that TLR2 confers detrimental immunity to HSV infection.

Considering that TLRs detect multiple PAMPs it is likely that multiple TLRs can act together to provide a comprehensive immune responses to a specific viral infection. In the case of HSV this appears to be the case. TLR2 detects an unknown molecule from the HSV viron, while TLR9 detects CpG [97]. Consequently it has been found that TLR2 and TLR9 function synergistically to respond to HSV infection in the brain [97]. HSV loads in the brain were found to be greater in the TLR2/9 double knockouts compared with brains from either the single knockouts. The expression of TNFα and CXCL9 in response to HSV were also dependent on TLR2 and TLR9. So while TLR2 in isolation might contribute to the pathogenesis of HSV, mediated by the release of cytokines and chemokines, TLR2 and TLR9 together appear to be required for an effective immune response to HSV, particularly in the brain [97]. Further evidence in support of a role for TLRs in response to HSV infection came from the observation that MyD88 knockout mice develop lethal encephalitis following intranasal infection with HSV [97]. Therefore TLR2 and TLR9 function together to confer resistance against HSV infection in the brain.

To date most studies concerning TLRs and HSV have used mice and there is a lack of human data in this area. However one study has reported that TLR3 is important in the human response

to HSV induced encephalitis (HSE). In this study it was found that two children with a heterozygous mutation in TLR3 were specifically predisposed to HSE [98].

The relevance of TLR3 in conferring immunity against HSV in humans is supported by the finding that alterations in signalling proteins downstream of TLR3 predispose to HSE. It has been reported recently that a young adult with a childhood history of HSE harboured a mutation in TRAF3. This mutation, a C to T at nucleotide 352 in exon 4, resulted in the substitution of a tryptophan for an arginine at position 118 (R118W). This mutation resulted in loss of TRAF3 expression and behaved in an autosomal dominant fashion. This mutation impaired TLR3 induction of IFN. Therefore the immunity mediated by TLR3 against HSV-1 in the CNS is dependent upon TRAF3 [99]. Interestingly the young adult described is otherwise healthy and critically has normal resistance to other viruses. It has been proposed that the weak yet detectable TRAF3 expression in this individual account for survival into adulthood [99]. Other TLRs that are implicated in conferring protective immunity against HSE in humans, are TLR 7, 8 and 9, since an autosomal recessive mutation in UNC93B, which is required for the proper signalling of these TLRs, also predisposed to HSE [100] (Table 3).

8.3 TLRs in response to bacterial meningitis

Meningitis or inflammation of the meninges can be caused by a wide variety of bacteria. The TLR response to bacteria that can cause meningitis has been evaluated in a number of mouse models of this disease. The gram positive bacteria group B streptococcus (GBS) is a major cause of meningitis in neonates. *In vitro* studies showed that heat inactivated GBS and a secreted factor

from GBS induced neuronal apoptosis via the TLR2 and MyD88 dependent production of NO from microglia. This observation is indicative of a mode of neurodegeneration that may contribute to the disease process of GBS meningitis in neonates [17]. Furthermore GBS can trigger microglial apoptosis in a pathway dependent upon TLR2 and caspase 8 [101]. A functional interaction between TLR2 and GBS is consistent with other studies which indicate that TLR2 is required for host defence against GBS in other disease settings such as arthritis and sepsis [102].

In a murine meningitis model using *streptococcus pneumoniae*, it was demonstrated that mice lacking TLR2 showed an earlier time of death compared with WT mice. When compared with the WT mice the TLR2 knockout mice had greater bacterial loads in the brain, indicating that TLR2 conferred protective immunity against *streptococcus pneumoniae* infection in the CNS [103, 104].

Citrobacter koseri is an example of a gram negative bacteria which causes meningitis in neonates. The contribution of TLRs to immunity against this pathogen was also evaluated in mice. It was shown that microglia from both TLR4 and MyD88 KO mice showed reduced proinflammatory cytokine responses to *C. koseri* compared with WT microglia, suggesting that the inflammatory response of microglia to *C. koseri* is dependent upon TLR4 and MyD88 [105] (Table 2).

The presence of polymorphisms in the TLR system affecting meningitis susceptibility in humans suggest that TLRs might be important in this illness, in particular those caused by *mycobacterium*

tuberculosis. It was found that a polymorphism in Mal C558T, and a TLR2 ploymorphism, T597C, is associated with susceptibility to meningitis caused by TB [106, 107] (Table 3). However such data is limited and further work is required in this area to determine the contribution of TLRs to human meningitis.

9. Future perspectives

In the past decade huge advances have been made in understanding innate immunity and there is a growing appreciation that the innate immune system is important in the CNS. In particular there is accumulating evidence that TLRs contribute to the pathogenesis of diseases of the CNS as eliciting their signalling pathways can be detrimental and lead to neurotoxicity. Conversely it is now known that triggering TLR signalling in the appropriate context in the CNS can confer neuroprotection and is therefore beneficial in some disease circumstances such as stroke.

It may be possible to treat neurodegenerative disorders in the future with drugs that stimulates neurogenesis from endogenous NPCs. For example in ischaemic models it has been shown that new neurons are not only generated but also functionally integrate and enhance cognative function which suggests that the use of drugs that trigger neurogenesis may be beneficial in stroke [21]. Alternatively the introduction of transplanted NPC from transdifferentiated stem cells from non-neural tissue such as the skin may be used. The use of NPC transdifferentiated from the patient's own non-neural stem cells to generate new neurons would avoid problems associated with host-versus-donor rejection [21]. In addition to this much work will be required to

understand how new neurons survive and integrate in order to be properly functional, therefore the processes following neuronal generation may be manipulated therapeutically in diseases of the CNS [21]. The finding that TLRs modulate adult neurogenesis [108] suggests that TLR manipulation may, in the future, be beneficial in therapies aimed at enhancing neurogenesis.

It is hoped that a more complete understanding of TLRs and their signalling pathways in the CNS will lead to the development of more effective treatments for a number of CNS diseases such as stroke, Alzheimers, MS and infectious diseases. However in order for this to occur better tools to study human TLRs are needed. There is a requirement for commercially available reliable and specific antibodies to human TLRs and their signalling proteins [19, 109]. The availability of such specific antibodies would allow for careful analysis of TLR expression and function. In addition better and more relevant animal models of human CNS diseases are required, especially those which take into account co-morbities and address age related factors of disease development such as Alzheimer's and stroke [59]. Furthermore an area that remains poorly understood is the role of negative regulators of TLR signalling in CNS diseases [19].

One of many concerns regarding the usefulness of mice models to human brain diseases where TLRs are implicated, is the fact that the expression pattern of TLRs differs between both species, with some striking examples. For instance and as stated above, human astrocytes express only TLR3 [10], while murine astrocytes express TLR2, 4, 5 and 9 mRNA [11]. Therefore the use of mouse models will have to be complemented by the use of relevant human cell and tissue culture models of CNS diseases [59].

Since TLRs are implicated in the pathogenesis of a wide range of CNS diseases, the development of therapeutics that target TLRs and their associated signalling pathways may be useful in the treatment of more than one condition, and increases the attractiveness of these PRRs to manipulation. However the manipulation of TLRs will have to be carefully considered. For example the potential use of TLR inhibitors in the treatment of conditions such as neuropathic pain may have unforeseen consequences on the influence that TLRs have in neuroprotection. Conversely stimulating TLRs to enhance immunity to infectious diseases may lead to neuroinflammation and neurotoxicity. Therefore the complexity of homeostatic roles that TLRs possess presents a huge challenge to their possible therapeutic manipulation. Nevertheless it is anticipated that further discoveries will lead to a better understanding of the role of TLRs in non infectious and infectious diseases of the CNS which may lead to better treatments for these conditions.

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The authors have nothing to disclose.

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Abbreviations Used

AD: Alzheimer's disease

ALRs: Aim2 like receptors

A*β*: Amyloid*β*

BBB: Blood brain barrier

CARD: caspase activating and recruitment domain

CB: Cerebral malaria

CFA: Complete freunds adjuvant

CNS: Central nervous system

CpG: Cytosine phosphate guanosine

DAMPs: danger associates molecular patterns

DCs: dendritic cells

dsRNA: Double-stranded RNA

EAE: Experimental Autoimmune Encephalitis

EGF: Epidermal growth factor

ERK: extracellular signal-regulated kinase

FADD: Fas-associated death domain

FAβ: fibrillar Aβ

FPRL1: Formyl peptide receptor-like 1

GBS: Group B streptococcus

GPI: Glycosylphosphatidylinositol

HMGB1: High mobility group box 1 protein

HSE: Herpes simplex virus induced encephalitis

HSP: Heat shock proteins heat shock proteins

HSV: Herpes simplex virus

IL-1β: Interleukin-1β

IL-6: Interleukin-6

iNOS: inducible nitric oxide synthase

IRAK: IL-1 receptor–associated kinases

IRF: Interferon Regulatory Factor

JNK: c-Jun N-terminal kinase

KO: Knockout

LPS: Lipopolysaccharide

Mal: MyD88 adaptor-like protein

MAPK: Mitogen-activated protein kinase

MMPs: Matrix metalloproteinases

MS: Multiple sclerosis

MyD88: Myeloid differentiation factor 88

NACHT: domain present in NAIP, CIITA, HET-E, TP-1

NAP1: NAK-associated protein 1

NFκB: Nuclear factor κB

NLRs: Nod-like Receptors

NO: Nitric oxide

NPCs: Neural progenitor cells

PAMP: Pathogen-associated molecular

Pattern

polyI:C: polyinosine-deoxycytidylic acid

PRRs: Pattern recognition receptors

RIP1: receptor interacting protein 1

RLRs: Rig like Receptors RLRs

ROS: Reactive oxygen species

SARM: Sterile α-and armadillo-motif containing protein

SINTBAD: Similar to NAP1 TBK1 adaptor

TAK1: transforming growth factor-b-activated protein kinase 1

TANK: TRAF family member-associated NFκB activator

TBK1: TANK-binding kinase

TIR: Toll/IL-1 receptor

TLR: Toll-like receptor

TNFα: Tumor necrosis factor α

TRADD: TNF-R-associated death domain

TRAF6: tumor necrosis factor receptor associated factor 6

TRAM: TRIF-related adaptor protein

TRIF: TIR domain-containing adaptor

VEGF: Vascular endothelial growth factor

WT: Wild type

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Figure 1. Families of Pattern recognition receptors (PRRs)

Innate immunity is composed of a number of PRRs and these include Toll-like receptors (TLRs), Nod like receptors (NLRs), Aim2 like receptors (ALRs) and Rig like receptors (RLRs). TLRs expressed on the cell surface sense bacterial products while endosomal TLRs sense bacterial and viral nucleic acids resulting in the expression of proinflammatory cytokines and type 1 IFN. Bacterial products are also sensed by the cytoplasmic NLRs, Nod1 and Nod2 leading to proinflammtory cytokine expression. NALP3, another member of the Nod family and a component of the inflammasome, detects bacteria in addition to environmental toxins and ATP to activate caspase 1. This results in the cleavage of pro-IL-1 to IL-1, leading to its release. Aim2 also activates caspase 1 following the sensing of cytoplasmic DNA, to permit IL-1 release. IFI16, another member of the ALRs, also senses cytoplasmic DNA, however unlike AIM2, IF116 activates IFN β expression. RLRs such as MDA5 and RIGI sense cytoplasmic viral RNA to activate type 1 IFN expression via the adapter MAVS.

Figure 2. Signalling from cell surface TLRs

Cell surface TLRs such as TLR4 and TLR5 and heterodimers of TLR1/2 and TLR2/6 detect bacterial products to trigger the TLR intracellular signalling pathways. Activation of TLRs results in the recruitment of the respective adaptors to the cytoplasmic region of the receptors. Recruitment of MyD88 results in the activation of IRAK4 and the phosphorylation of IRAK1. IRAK1 can then bind TRAF6 (not shown). IRAK2 promotes the ubiquitination of TRAF6 that results in the recruitment of the TAB2/3 complex and the activation of TAK1. This leads to the activation of the IKK complex and the movement of NFκB into the nucleus. The IKK complex is also involved in the activation of TPL2 and the stimulation of the ERK MAP kinase pathway via MKK1/2. The ERK pathway contributes to c-FOS and JUN activation, subunits of AP1. In addition TAK1 triggers the activation of the p38 and JNK pathways via MKK3/6 and MKK4 respectively to activate AP1. Activation of NFκB and the MAP kinase pathways results in the expression of proinflammatory cytokines. TLR4 activation by LPS also results in the internalisation of TLR4 to endosomes. Here TLR4 engages with the adaptors TRAM and TRIF to trigger TRAF3 dependent activation of the IKKε/TBK1 complex, which also contains DDX3. Activation of this complex results in the phosphorylation and dimerisation of IRF3 and IRF7, resulting in their movement into the nucleus and the expression of type 1 interferon.

Figure 3. Signalling from endosomal TLRs

Microbial nucleic acid engagement with the endosomal TLRs triggers the NFκB and MAP kinase pathways similar to the cell surface expressed TLRs as detailed in figure 2. In addition to those pathways TLR7/8 and TLR9 activate the IRAK1/TRAF6 complex via MyD88 and IRAK4. This leads to the activation of IKKα resulting in the phosphorylation and dimerisation of IRF7. These IRF7 dimers translocate into the nucleus to trigger type 1 IFN production.

Table 1: Comparison of TLR mRNA and protein expression in human and mouse cells of the CNS

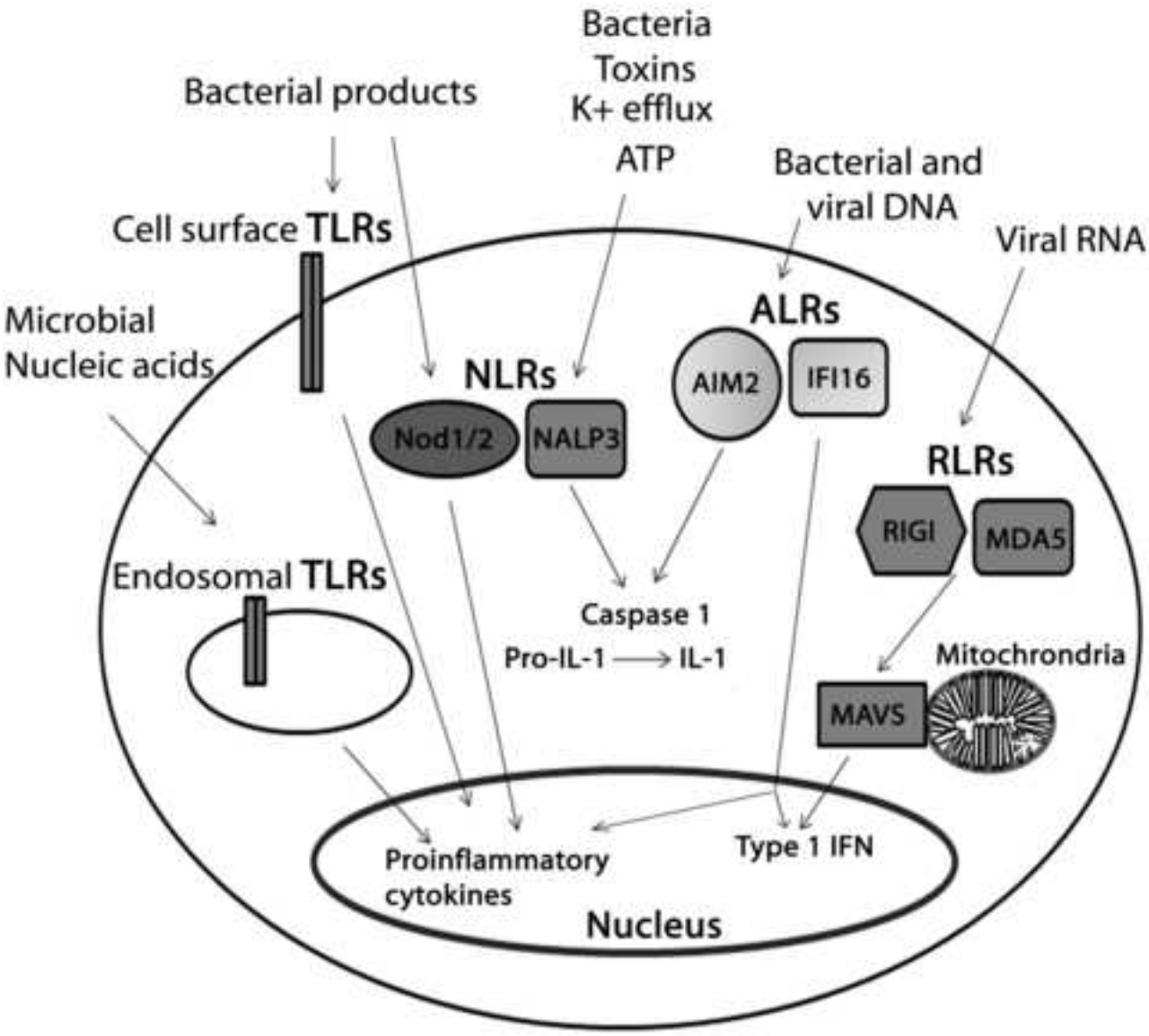
Table 2: Evidence from knockout mice that TLRs are involved in CNS diseases

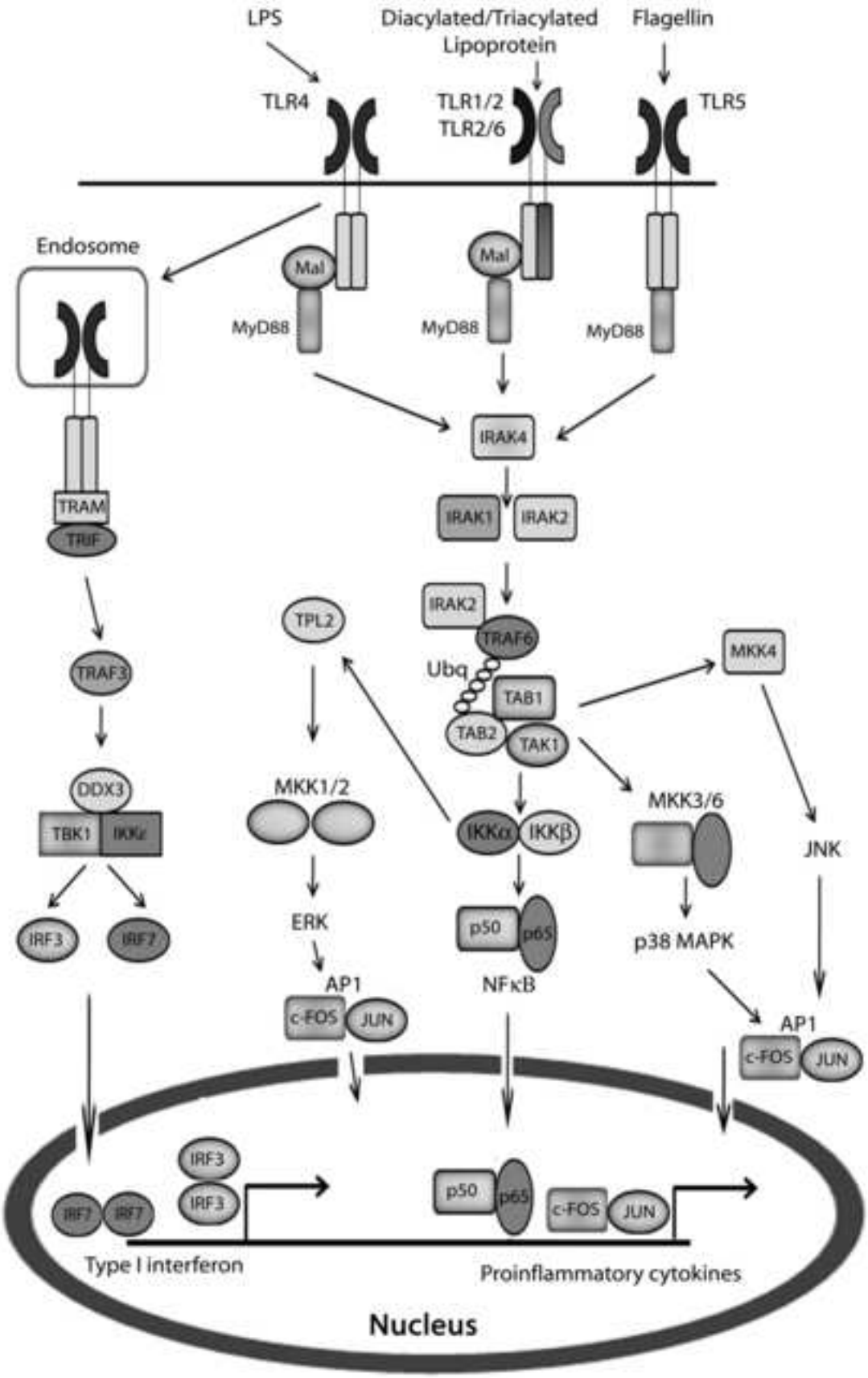
Table 3: Evidence from human studies implicating TLRs in diseases of the CNS

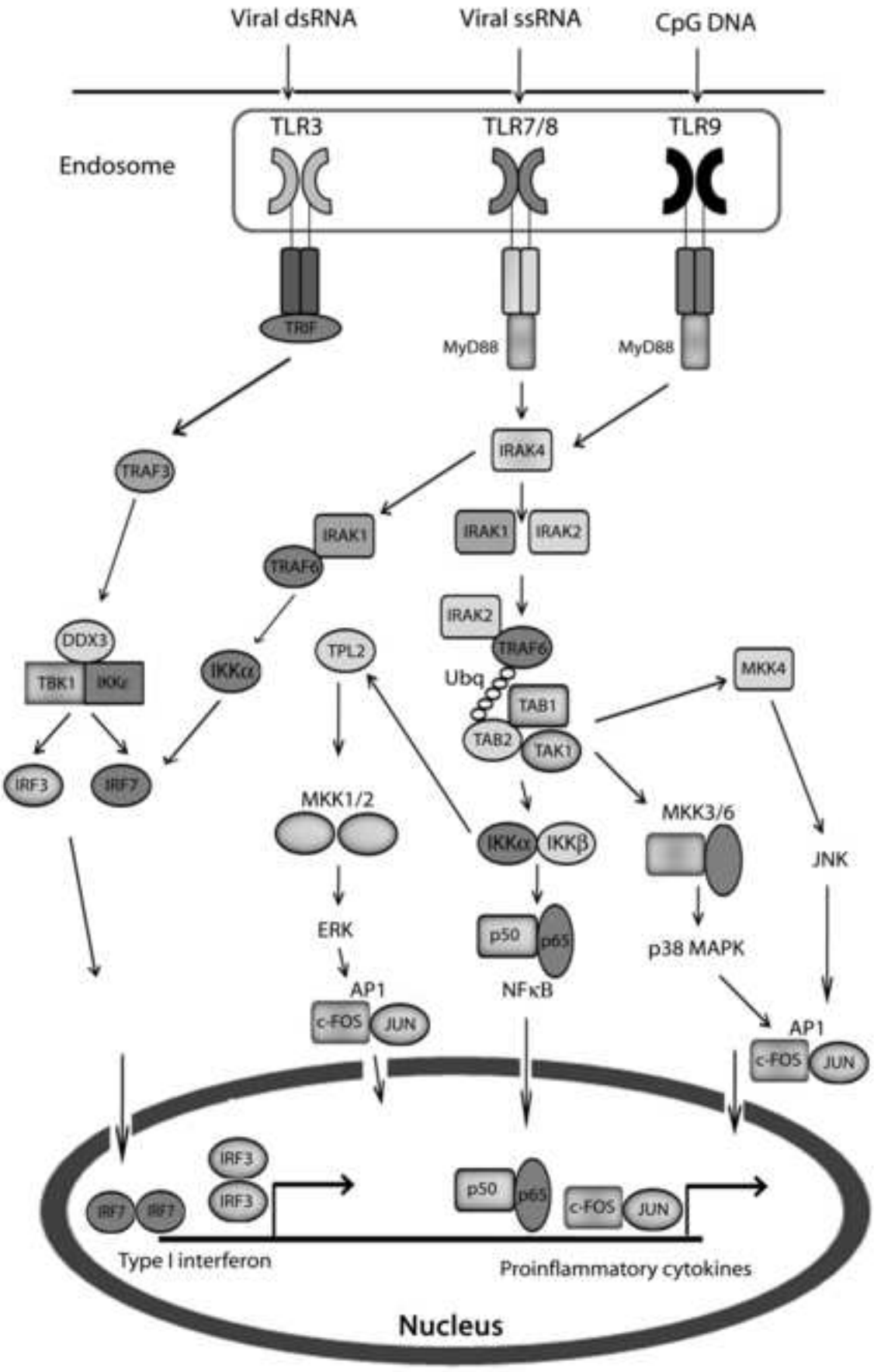
Species	Human	mouse	Human	mouse
Molecule Detected	**mRNA**	**mRNA**	**Protein Immunoreactivity**	**Protein Immunoreactivity**
TLR1	Microglia [16] Astrocytes [16, 110]	Astrocytes [111] Microglia [15]		
TLR2	Microglia [16] Astrocytes [110]	Microglia [15] Astrocytes [11, 111] Neurons [12]		Neurons [12, 13]
TLR3	Microglia [16] Astrocytes [10, 110] Neurons [112]	Astrocytes [111] Microglia [15] Neurons [12]	Astrocytes [58] Neurons [112]	Neurons [12]
TLR4	Microglia [16] Astrocytes [16, 110]	Microglia [15] Astrocytes [11, 111] Neurons [12]	Astrocytes [58]	Neurons [12, 13]
TLR5	Microglia [16] Astrocytes [16]	Microglia [15] Astrocytes [11, 111]		
TLR6	Microglia [16]	Astrocytes [111] Microglia [15]		Neurons [13]
TLR7	Microglia [16]	Astrocytes [111] Microglia [15]		Neurons [13]
TLR8	Microglia [16]	Astrocytes [111] Microglia [15]		Neurons [13]
TLR9	Microglia [16] Astrocytes [16]	Microglia [15] Astrocytes [11, 111]		

Disease	**TLR Implicated**	**Beneficial or Detrimental**
Non infectious diseases: Alzheimers Disease	TLR2, TLR4, CD14 [39]	Both beneficial and detrimental
Experimental Autoimmune Encephalitis	MyD88 [54] TLR4 [113] TLR9 [49, 113] TLR2 (expressed on B cells) [57] TLR4 (expressed on B cells) [57]	Detrimental Beneficial Controversial Beneficial Beneficial
Stroke	TLR4 [62] TLR2 [64]	Detrimental Detrimental
Glioma	TLR9 [71]	Beneficial
Infectious Diseases: Cerebral Malaria	Multiple TLRs [89-91]	Controversial
Herpes Simplex Encephalitis	TLR2 [93, 94] TLR2&TLR9 [97]	Detrimental Beneficial
Bacterial meningitis	TLR2 [103, 104] TLR4 [105]	Beneficial Beneficial

Disease	TLR Implicated	Detrimental or Beneficial
No Infectious diseases: Alzheimers	TLR4 polymorphism Asp299Gly [37] CD14 increased in senile plaques [34]	Beneficial Unknown
Multiple Sclerosis	Increased TLR3 and TLR4 [58]	Unknown
Stroke	TLR4 polymorphism C119A [114]	Detrimental
Glioma	TLR9 expression evaluated [72] Phase I clinical trial [75] Phase II clinical trial [76]	Beneficial
Infectious diseases: Cerebral Malaria	TLR9 polymorphisms [92]	Unknown
Herpes Simplex Encephalitis	TLR3 polymorphism P554S [98] TRAF3 polymorphism R118W [99] UNC-93B polymorphism (TLR3, 7, 8, 9) [100]	Detrimental Detrimental Detrimental
Bacterial meningitis	MAL polymorphism C558T [106] TLR2 polymorphism T597C [107]	Detrimental Detrimental







*Graphical Abstract

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