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TLRs as therapeutic targets in CNS inflammation and infection

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1. ABSTRACT

Far from being an immune-inert site, the cells of the CNS, including microglia, astrocytes and neurons, express an abundance of innate immune receptors, including toll like receptors (TLR), which recognize conserved structural motifs in pathogens. These TLRs have been implicated in both infectious and non-infectious CNS pathology and appear to play important roles in both tissue surveying and repair. Therapeutics that modulate their activity are being explored for the treatment of infectious and inflammatory diseases, cancer and neurodegenerative processes.

2. THE CNS, A UNIQUE IMMUNOLOGICAL ENCLAVE

Extensive literature has shown that immune cells contain the receptors, peptides, neurotransmitters and signaling pathways required to respond to neural factors. These factors are generally associated with the cells in the CNS. In turn, despite their mostly non-immune cell functions the CNS cells (neurons, astrocytes, oligodendrocytes and microglia) are equipped with pattern recognition receptors (PRR) that are usually associated with the innate immune system and can initiate immune responses to pathogens (1-3).

Neurons transmit informatic signals via the propagation of action potentials and transynaptic release of neurotransmitters (4). Astrocytes support critical metabolic functions of neurons by storing and catabolizing glycogen as well as glutamate, and by regulating ion homeostasis (5). Oligodendrocytes are critical nodal cells that synthesize the myelin (white matter) sheath that insulates axons in the CNS (6). Only microglia, which are the closest cells the CNS has to classical bone-marrow-derived immune cells seen in the periphery, have immune surveillance of the local microenvironment and maintenance of neuronal homeostasis as a primary function (7).

Pattern recognition receptors are expressed predominantly by astrocytes and microglia, but have been identified in neurons as well, and are upregulated under conditions of stress or injury. These receptors identify a broad spectrum of evolutionarily conserved lipids, glycolipid, lipopeptide, protein and oligonucleotide motifs and function cooperatively to initiate and tailor inflammatory, innate and adaptive immune responses to infectious agents. Moreover, these receptors, and others more recently recognized such as the danger associated molecular pattern (DAMPs) receptors, appear to be key to the responses to brain injury, ischemia, autoimmune and neurodegenerative processes (8-10).

This review will discuss the emerging evidence that Toll like receptors (TLR) - and other less-well characterized innate immune receptors - play a key role in responding to infectious agents, maintaining homeostasis in the CNS and modulating the neurodegenerative response to injury leading to the hypothesis that TLRs could be effective therapeutic targets to treat a variety of CNS inflammatory and infectious processes.

3. INNATE IMMUNE RECEPTORS: PAMPS AND DAMPS

Innate immune receptors include several families of evolutionarily conserved molecules that directly detect highly conserved pathogen-associated molecular patterns (PAMPs) upon microbial invasion or tissue damage and initiate a biological response. PRRs include both membrane-bound and intracellular proteins. They include Toll-like receptors [TLRs], Nod-like receptors [NLRs], RIG-1 like helicases [RLHs], scavenger receptors and soluble molecules like collectins, mannose binding lectin (MBL) and members of the pentraxin (PTX) family (e.g., CRP and PTX3). They are expressed mainly by cells involved in immune reactions such as dendritic cells, macrophages, granulocytes, T cells, B cells, natural killer [NK] cells, and mast cells, as well as by endothelial and epithelial cells, and are mostly involved in recognition and response to microbial compounds, although some endogenous targets have been identified (9).

In contrast, DAMP receptors comprise a heterogeneous group that respond to endogenous danger signals such as high mobility group box 1 protein (HMGB-1), heat-shock proteins (HSPs), uric acid, altered matrix proteins, and S100 proteins, and mediate inflammatory responses through the receptor for advanced glycation end-products (RAGE) (8, 11, 12). A wealth of data suggests that these receptors and their signaling are highly integrated. For example, TLRs and NLRs cooperate to induce pro-IL-1-beta and IL-18 production and prime Caspase I in response to bacterial products and products of damaged cells (13). Of the innate immune receptors, the TLR family are the best characterized to date.

4. TOLL-LIKE RECEPTORS

TLRs were first discovered in drosophila by the resistance they lent against fungal infections (14). In mammals, TLRs are expressed either on the cell surface (TLR1, 2, 4, 5, 6 and 11) or in intracellular compartments like endosomes (TLR3, 7, 8 and 9) (15). TLR2 recognize peptidoglycan, triacyl lipopeptides lipoteichoic acids and other bacterial cell wall components, often by forming heterodimers with TLR1 and TLR6, as well as zymosan in fungi (together with Dectin 1) and endogenous proteins HMGB1 and HSP70. TLR5 binds flagellin and TLR4 responds to lipopolysaccharide in gram negative bacteria as well as a host of other endogenous and exogenous ligands (taxol, peptidoglycan, heat shock protein 60 & 70, beta-defensins, etc.). TLR11 recognizes profilin-like molecules derived from protozoa, and perhaps PAMPs from uropathogenic *E. coli*. In addition, TLR3, 7, 8, and 9 usually reside inside the cell and are activated by nucleic acids. TLR3 responds to double stranded RNA, TLR7 and TLR8 to single stranded RNA and TLR9 to DNA rich in non-methylated cytosine-guanosine (CpG) motifs. Lastly, there remain some orphan family members, including TLR10 with no known ligands or function as of yet (16). The full spectra of ligands can be found in a recent review (17).

TLRs share a basic structure with an N-terminal ectodomain composed of leucine-rich repeats (LRRs) and cytoplasmic Toll-IL-1 receptor (TIR) domain that recruits adaptor molecules to initiate downstream signaling (18). Their signaling is extremely complex and has been reviewed extensively elsewhere (9). Briefly, recruitment of adapters (the most prominent being MyD88) leads to the formation of a complex with interleukin-1-receptor (IL-1R)-associated kinase-4 (IRAK-4), IRAK-1, tumor necrosis factor-receptor associated factor 6 (TRAF6). These ultimately trigger NF-κB, p38, and ERK activation and increased transcription of proinflammatory cytokines such as TNF-alphą IL-6 and IL-12, or otherwise the activation of IRF7, which results in the upregulation of type I interferons (IFN) (9, 19, 20). Together they initiate a localized inflammatory response and function as a first-line defense against pathogen invasion (21, 22). In the periphery TLRs are located on antigen presenting cells such as B cells, dendritic cells, and monocytes/macrophages. In the CNS, these receptors are strategically expressed at or near the blood brain barrier, around the ventricles and choroidal plexus, which lack effective blood brain barrier and are therefore more exposed to pathogens. They are also expressed in cells of the brain parenchyma including microglia, astrocytes, oligodendrocytes, and neurons (23–26).

5. TLRS IN THE CNS

The brain had been classically viewed as an immune privileged organ due to the absence of circulating lymphocytes. This was mostly due to the properties of the blood brain barrier (BBB) that impedes the passage of large molecules from the periphery into the brain and limits the circulation of immune cells. During the past decade, several studies have demonstrated that microglia and astrocytes are the resident immune cells of the brain capable of orchestrating a full response to pathogens (27, 28). Expression of TLR1-10 was shown in microglia, whereas astrocytes robustly express TLR3 levels, but low-levels of TLR1, 4, 5, and 9, and rare-to-undetectable TLR2, 6, 7, 8, and 10 mRNA (29, 30). Oligodendrocytes also express a limited set of functional TLRs (TLR2 and 3) (31). The expression of TLR in neurons appears to be similarly restricted as only TLR3, TLR4 and TLR8 have been reported (32, 33). Of interest, new thought-provoking studies assessing the expression and function of TLR3 and 8 in the neuronal growth cones suggest that TLRs could play a developmental role in controlling neurite growth and maturation in the CNS (25, 32, 34).

Upon trauma or infection, glia and astrocytes function in an autocrine and paracrine manner rapidly upregulating TLR and other innate immune receptors (IIR) and producing the pro-inflammatory cytokines and chemokines responsible for local inflammation and eventually cellular infiltration (35, 36). As mentioned above, the effects of TLR agonists in CNS appear to be dual: On one hand, administration of TLR agonists induces localized production of inducible nitric oxide synthase (iNOS), numerous cytokines (IL-6, IFN-gamma, TGF-beta, GM-CSF, BAFF, IL-1-beta and TNF-alpha) and chemokines (CCL2, CCL5, CCL20, CXCL10, CXCL11, CXC1 and CX3CL1). These in turn lead to reduced glutamate uptake, increased connexin 43 expression and function, increased BBB permeability, activation of neighboring endothelial cells, glia and astrocytes, and recruitment of peripheral monocytes and macrophages (36-38). On the other, these responses may be tempered by increased production of neurotropic and neuroprotective factors such as nerve growth factor (NGF), brain derived growth factor (BDGF), leukocyte migration inhibitory factor (LIF), and vascular endothelial growth factor (VGEF), which reduce astrocytosis and improve neuronal survival (33, 39, 40). The balance may be aided by an apparent compartmentalization of the response that prevents widespread inflammation in response to a localized trigger, and the route of administration appears to be determinant as to the overall effect. For example, while systemic administration of TLR ligands can induce a mild proinflammatory milieu (41), direct inoculation in the CNS of CpG ODN (TLR9 ligand) or LPS (TLR4 ligand), results in acute encephalitis, astrocytosis, and can result in death (36, 42, 43). Of note, in newborn mice, administration of TLR9 ligands intranasally results in the immediate upregulation of proinflammatory cytokines such as IL-1, TNF-alpha and IFN-gamma in the olfactory bulb which lacks a functional blood brain barrier (Pedras-Vasconcelos, Tonelli and Verthelyi, unpublished) but no overt *in vivo* effects were observed. Lastly, CNS TLRs can be triggered by endogenous ligands. Several reports suggest that patients with Alzheimer's disease and Parkinson's disease may have elevated levels of endogenous TLR ligands, such as HMGB1 compromising the balance between neuroinflammation and neuroprotection(40).

6. THERAPEUTIC APPLICATIONS FOR TLR AGONISTS IN CNS

To date, TLR7 agonist Imiquimod is licensed as a topical treatment of anogenital warts, actinic keratosis and superficial basal cell carcinoma. Several other agonists and antagonists of TLR4, 7, 8 and 9 are being actively pursued as vaccine adjuvants and for the treatment of infections, autoimmune diseases, allergies and cancers (44, 45). Specifically in CNS, there are data indicating that TLR could be targeted in the management of acute and chronic neurological diseases (3, 10, 40, 46).

6.1. Immunoprotection

Modulators of the innate immune response provide an attractive approach to limiting the spread and pathogenicity of neurotropic infections. The ability of TLR agonists, CpG ODN in particular, to activate the innate immune system and improve its response to a wide variety of pathogens has been amply demonstrated in rodent models and the general mechanism by which the response is improved has been largely elucidated (21, 47). The immunoprotective potential of CpG ODN has been demonstrated by our group in primates as rhesus macaques challenged with *L. major* had significantly reduced lesions, accelerated healing, and lowered parasite loads when treated with CpG ODN (48, 49). Further, the protective effect extended to immunocompromised macaques suggesting that the effect did not require an intact adaptive immune system (50). Interestingly, those studies also showed that unlike mice, in primates the response to CpG ODN is sequence, backbone, and structure dependant as primates treated with CpG ODN type D, but not type K, had improved outcome (49, 51, 52).

Several lines of evidence suggest that TLRs play a key role both in the pathology and the immune response to neurotropic pathogens (53-55). CNS infection upregulates the expression of TLR in microglia, and astrocytes as well as increases the permeability of the BBB allowing for infiltrating leukocytes (56-58). As a result, there is a net increase in TLR expression in CNS. Their protective role is underscored by studies in knock-out (KO) mice. For example, absence of TLR2 or 4 increased the susceptibility to infection and accelerated death in mice challenged intra-cerebrally with *Streptococcus pneumoniae* (59). Likewise prion disease progression is accelerated in mice lacking TLR4 (60), mice lacking MyD88 have higher susceptibility to Herpes Simplex virus (HSV)-1 (61), and $IRF7^{-/-}$ mice have a blunted IFN response to West Nile virus (62).

In contrast, in other models, TLRs appear to play important roles in disease pathogenesis: for example, TLR3-mediated induction of TNF-alpha and IFN-beta

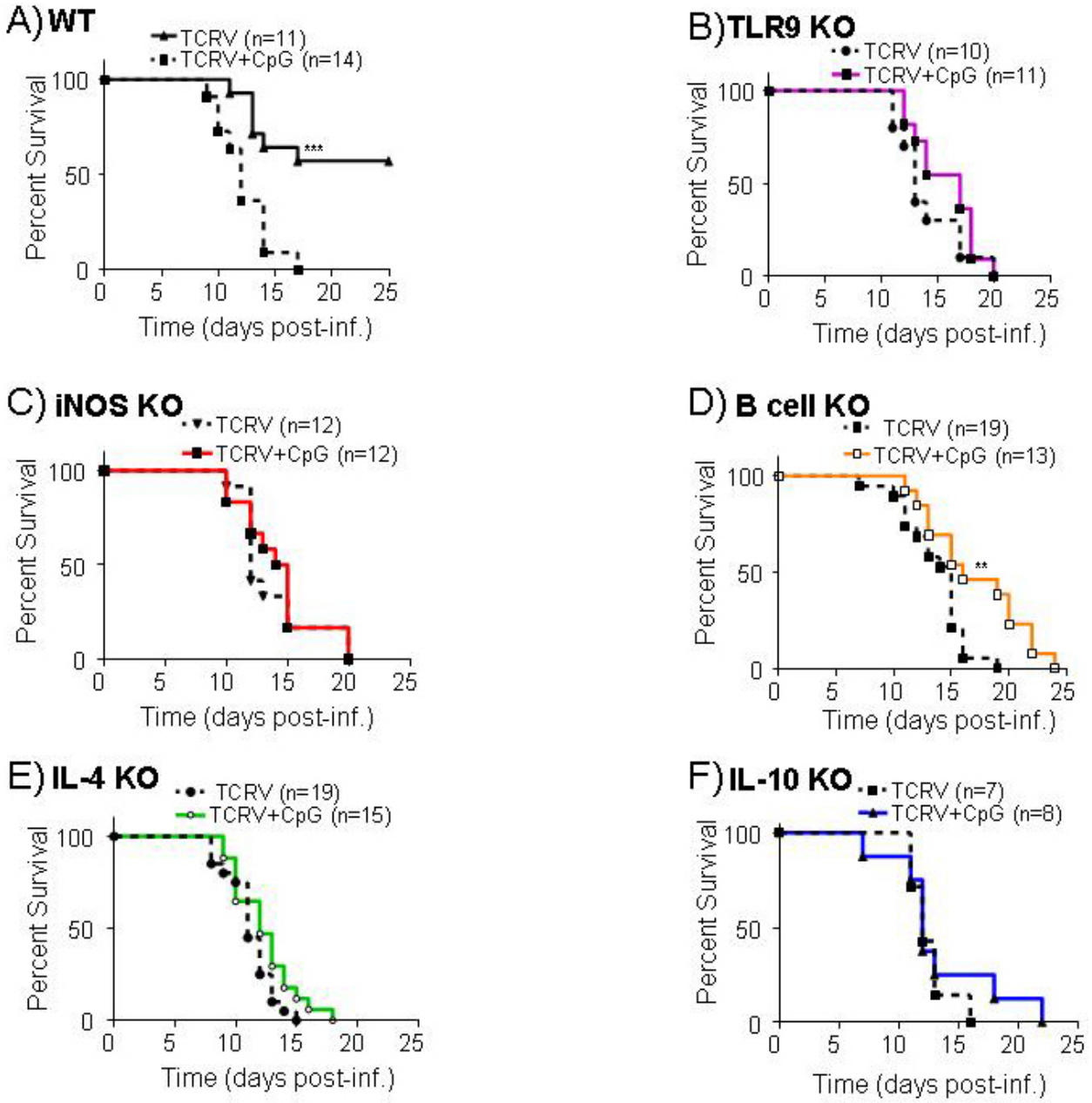

Figure 1. Survival of TCRV-infected mice untreated or treated with CpG ODN. One to 3 day old mice [(A)WT, (B) TLR9 KO, (C) iNOS KO (D) B cell KO (E) IL-4 KO and (F) IL-10 KO] were challenged i.p. with 2000 $TCID_{50}$ of TCRV and treated with CpG ODN (50µg/mouse, i.p.)((68) and Pedras-Vasconcelos and Verthelyi, unpublished).

enhances West Nile and rabies virus access to the CNS respectively (63, 64). In models of cerebral malaria, the role of TLRs is controversial as Coban *et al.* showed that mice lacking TLR2, TLR9 and MyD88 had reduced CNS infiltration of $CD8^+$, $CCR5^+$ T cells and of $CD11c^+$ dendritic cells, including the $CD11c^+$, $NK1.1^+$ and $B220^+$ subsets. In addition they also observed the up-regulation of genes such as Granzyme B, Lipocalin 2, CCL3 and CCR5, and proposed that the absence of these receptors resulted in improved survival. Other groups, however, could not confirm a deleterious role for TLR2 or TLR9 (65-67).

In other models, the absence of TLR9 did not modify the clinical progression. For example, in a model of arenavirus encephalitis, mice lacking TLR9 developed signs of encephalitis and died with similar kinetics as their wild type (WT) counterparts ((68) and Figure 1). Our laboratory used this arenavirus model to explore the role of TLR in inducing CNS proinflammatory cytokines to control viral replication in non-lytic viral encephalitis. Neonatal mice challenged with Tacaribe Arenavirus (a model for encephalitic South American Arenaviruses, which are Class 1A pathogens) develop a lethal

meningoencephalitis characterized by high IFN-gamma and TNF-alpha levels and mild T cell infiltration (68, 69). Despite being present in low levels, T cells play an important role in pathogenesis as several studies show increased survival in challenged mice that lacked T cells (70-72). In this model, systemic administration of immunostimulatory CpG ODN at the time of infection accelerated antibody production to the virus, and reduced inflammation and T cell infiltration as evidenced by reduced levels of IFN-gamma, TNF-alpha and chemokines CCL5/RANTES and CXCL10/IP10 in the CNS, resulting in improved survival (30-50%) (68). In contrast, administration of TLR7/8 stimulating R848/Resiquimod failed to protect the mice. CpG ODN improved survival was dependent of the local expression of TLR9, iNOS, IL-4 and IL-10 and on the presence of mature B cells, ((68) and Figure 1). Importantly, controlling the local inflammatory response to the virus and the oligonucleotides by treating mice systemically with a mAb to TNFα resulted in 100% survival and complete viral clearance avoiding a chronic inflammatory insult and possible neurodegenerative sequelae (69). These studies underscore the need to that effective therapy for CNS infections may require activating the innate immune system to eliminate the infection while curtailing the inflammatory response they trigger to protect the parenchyma.

The optimal TLR agonist for individual neurotropic viruses may vary. A recent study suggests that mice challenged with HSV-2 had improved survival and reduced local inflammation when treated with Poly I:C but not LPS or CpG ODN (73). It is interesting to note that in both viral models intranasal administration of TLR agonists resulted in effective protection. Studies performed by our group in collaboration with Dr Tonelli indicate that intranasal administration of TLR agonists may results in a marked increase in cytokine expression in the olfactory bulb, the consequences of which are still unknown.

Despite their differences, these models suggest that targeting TLR receptors may be an effective therapeutic strategy, however there remain serious limiting factors in translating these findings to effective therapies for humans. The first involves the development of delivery systems that allow for effective doses of TLR agonists to reach the CNS without causing systemic toxicities. The second relates to the differential cellular distribution and specificity of TLR in humans versus rodents which requires the development of more appropriate animal models (74, 75). A third, is the short-lived effects of TLR agonists, as both Poly I:C and CpG-ODN up-regulate mRNA within minutes, leading to a short-term increase in IFN and cytokine secretion. While the short innate immune burst may trigger an effective response, it restricts the therapeutic and prophylactic value of these innate immune response modulators (IIRMs). Indeed, this was evident in most of the infectious models investigated, as improved survival was evident only when treatment occurred before or shortly after infection. In fact, even when the CpG ODN were modified to have a prolonged half life, the therapeutic window was brief (47, 76). Lastly, recent studies by Wagner *et al*, suggest that repeated systemic exposure to TLR agonists result in sustained CNS activation (41) which may prevent their extended use during an infectious outbreak although its long term effects are yet to be explored.

6.2. TLRs and inflammation

Acute trauma to the CNS can result both in direct neuronal loss due to damage to the cell body of a neuron, and in Wallerian degeneration, where axons distal to the injury site degenerate due to lack of neurotrophic support (77). If damage is sufficiently severe and the resulting inflammation fails to be downregulated, secondary degeneration can take place (78). In this context, several reports indicate that TLR2 and 4 are upregulated in neurons under conditions of stress and mice lacking these receptors had reduced neuronal apoptosis (79, 80). However, the induction of innate immune responses in the CNS are not necessarily detrimental as they help to demarcate the area of insult restricting the influx of leukocytes, limiting astrocytosis and reducing neuronal death, and therefore protecting or restoring the homeostasis of the surrounding tissue(81, 82). In this context, some degree of neuro-inflammation may benefit the repair process (83, 84). Therefore, TLR agonists or antagonists could be targeted to limit damage and accelerate repair.

Experimental results with an optic nerve crush injury model in rodents suggest that injection of CpG at the time of nerve injury results in a significant increase in surviving retinal ganglion in the retina as well as axons within the optic nerve up to two weeks later (85). The effect correlates with enhanced infiltration of CD3+ T cells within the optic nerve injury site and may partly be due to the transient down-regulation of peripheral CD25+ CD4+ T regulatory cells that normally keep existing autoimmune T cells in check (84), but the full mechanism of protection is yet to be fully understood.

Modulation of the inflammatory response that exacerbates cerebral injury after ischemia is another attractive therapeutic target for IIRMs. Several studies have shown that TLR2 and TLR4-mediated production of proinflammatory cytokines plays an important role in amplifying the inflammation secondary to trauma as mice lacking functional TLR2 or TLR4 have smaller infarcts (80, 86). In contrast, preconditioning with lipopolysaccharide (LPS), or CpG ODN, reduces tissue damage and improves revascularization in the event of a subsequent cerebral ischemic brain injury (1-14 days following treatment) (87-89). The protection requires iNOS and eNOS and the activation of tumor necrosis factor receptor 1 (TNF-alpha R1) and/or type I IFNs. Studies in rats showed that administration of IFN-beta before or after (4 to 6 hours) stroke resulted in reduced neutrophil infiltration and BBB stabilization (90, 91). In addition, since microglia, astrocytes and neurons can produce type I IFNs in response to TLR3 activation, the use of Poly I:C analogs has been proposed treat cerebral ischemia (33). IFNβ has been brought to clinical trials as a potential therapy to reduce the infarct volume and improve reperfusion.

Lastly, inflammatory processes are known to play a key role in the progression of neurodegenerative diseases

such as Alzheimer's disease (AD). AD is the most common cause of dementia in elderly patients and is characterized by a loss in synaptic plasticity and progressive cognitive impairment. Histopathologically, it is characterized by the accumulation of plaques and neurofibrillary tangles containing tau protein (92). Convincing evidence is emerging that TLR play a role in protecting or enhancing the immune and inflammatory response to oligomerized beta-amyloid aggregates in AD (92). Studies in beta-amyloid precursor protein and presenilin-1 knock-in (APP/PS1KI) mice (a widely used model of AD) show increased expression of TLR2, 4, 7 and 9 in brain and spinal chord of mice 6 months of age when deficits in cognitive and motor performance become evident (93). The increased TLR expression may result in accelerated cellular uptake and clearance of beta-amyloid as suggested by studies in mice lacking functional TLR4, but may make also make neurons more susceptible to oxidative stress and apoptosis (92, 94).

6.3. TLR agonists and cancer

Although not the focus of this review, several TLR agonists have been explored as stand-alone cancer therapeutics based on the concept that their cytotoxic effects coupled with the enhanced antigen presentation may lead to improved immune responses to tumors. CpG ODN, Imiquimod and monophosphoril lipid A have been tested in animal models. Intratumoral administration of lipid A moiety of LPS to mice with highly lethal and treatment resistant glioblastoma multiforme (GM) reduced tumor size significantly in mice (95). The clinical improvement was reduced in animals lacking TLR4, and appears to be mediated by increased leukocyte infiltration (95, 96). Testing of TLR7 ligand in models of intracranial malignant melanoma showed that although imiquimod increased the survival of peptide-pulsed dendritic cells and $CD8^+$ priming leading to reduced tumor size, the increased hemorrhaging and inflammation-induced resulted in increased mortality underscoring the need to balance immune activation while retaining control, of the inflammatory process (97). In a different model, Carpentier *et al* showed that administration of CpG ODN prolonged survival in mice with neuroblastomas, partly by increasing local apoptosis (98). Further, a recent study by the same group recently showed variable levels of TLR9 expression in leukocytes infiltrating human gliomas suggesting that CpG ODN administration could have clinical benefit for these patients (99). Early results from a phase I clinical trial have not demonstrated the desired efficacy as only 2 of 24 patients showed minor therapeutic response highlighting once again the differential expression of TLRs both on immune cells and on tumor cells and the resulting limitations of the murine models (99). The beneficial or detrimental effect of TLR agonists may depend on the cancer type. This complexity is illustrated by studies showing that MyD88 KO mice are more resistant to induction of certain tumors and more susceptible to others (100, 101).

6.4. TLRs and autoimmunity

Failure to appropriately regulate self-responses triggered by IIR can have serious pathological consequences, as several TLRs have been linked to the development of autoimmune diseases (102) an area where therapies that target TLR are being investigated (103, 104).

Multiple sclerosis (MS) is a chronic debilitating autoimmune disease of the CNS characterized by infiltration by activated lymphocytes and phagocytes, proinflammatory cytokines, such as IL-17, IFN-gamma and GM-CSF, inflammation, and increased expression of TLR in microglia and astrocytes that lead to progressive demyelination and axonal/neuronal injury. The dual role of TLR is underscored by studies in experimental autoimmune encephalitis (EAE), the corresponding animal model, showing that while MyD88 KO mice are resistant to EAE, TLR9 and TLR2 KO mice are more susceptible (105-109). Moreover, individual TLR agonists, like LPS, may induce the proinflammatory cytokines that trigger the disease or facilitate myelin repair, depending on the model and the stage of the disease when they are administered (110, 111). Of note, MyD88-independent TLR3 stimulation with Poly I:C was found to suppressed relapsing demyelination and subsequent paralysis, in part through the induction of IFN-beta and CCL2 (112). IFN-beta is approved for the treatment of MS, as it decreases inflammatory lesion formation in the CNS, prolongs remission, and lowers relapse rate. Recent studies show that IFN-beta interferes with the generation of Th17 inflammatory T cells through STAT1 and IL-27 (113), and directly inhibits infiltrating myeloid-derived macrophages and DCs (114). IFN-beta may also reduce chemokine expression and myelin phagocytosis by microglia and decrease MHC class II upregulation (114). Direct effects of TLR3 agonists on astrocytes, oligodendrocytes and neurons can also not be ruled out.

7. SAFETY CONSIDERATIONS

There is now substantial clinical experience with TLR agonists as vaccine adjuvants and in the treatment of allergies, cancer and infectious diseases. The safety of systemic administration of TLR agonists has been addressed elsewhere (115-119). However, the delicate structures of the CNS make it particularly vulnerable to the inflammatory response generated by TLR agonist. Potential safety concerns include: First, the generation of unregulated non-specific inflammatory responses leading to permanent disruption of the CNS architecture. Studies show that systemic administration of CpG ODN resulted in increased TNF-alpha levels and sustained type I cytokine production in CNS (41), while *in situ* administration can result in an acute increase in proinflammatory cytokines such as IFN-alpha and the consequent development of fever, malaise, depression and other neurological symptoms. In a study in patients with glioblastoma, Carpentier *et al.* showed that intratumoral administration of CpG-28 (type K ODN) in a phase I clinical studies caused moderate adverse effects in 23 out of 24 patients mostly consisting of fever, worsening of neurological condition, and lymphopenia; one patient developed dose limiting toxicity events and did not recover (120). Second, systemic off target effects. These will be largely determined by the product delivery and the dose required toachieve therapeutic concentrations in CNS. Third, potential breaks

in tolerance and subsequent development of autoimmune disease (see above). Lastly, it is possible that the activation of immune associated genes may lead to the loss of normal neuronal functions, and resulting deficit may become permanent if the inflammation is chronic, as is the case with neuro-degenerative conditions such as Alzheimer's, or spongiform encephalopathies (121-123).

8. CONCLUSION

It is clear that a better understanding of the role and regulation of inflammation in the CNS will be necessary before we can safely utilize IIRMs to treat infectious, neurodegenerative or inflammatory diseases. However, emerging data suggests that the controlled induction or suppression of innate immune processes represents a viable therapeutic approach that should be thoroughly investigated.

9. REFERENCES

1. Sternberg E.M.: Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. *Nat Rev Immunol*, *6*, 318-328 (2006)

2. Quan N. & W.A. Banks: Brain-immune communication pathways. *Brain Behav Immunity*, 21, 727-735 (2007)

3. Okun E., K.J. Griffioen, J.D. Lathia, S.C. Tang, M.P. Mattson, & T.V. Arumugam: Toll-Like Receptors in Neurodegeneration. *Brain Res Rev*, Sep 12 (2008)

4. Kullmann D.M. & K.P. Lamsa: Long-term synaptic plasticity in hippocampal interneurons. *Nat Rev Neurosci*, *8*, 687-699 (2007)

5. Elward K. & P. Gasque: "Eat me" and "don't eat me" signals govern the innate immune response and tissue repair in the CNS: emphasis on the critical role of the complement system. *Mol Immunol*, 40. 85-94 (2003)

6. Kassmann C.M. & K.A. Nave: Oligodendroglial impact on axonal function and survival - a hypothesis. *Curr Op Neurol*, 21*,* 235-241 (2008)

7. Hanisch U.K. & H. Kettenmann: Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci*, 10, 1387-1394 (2007)

8. Foell D., H. Wittowski & J. Roth: Mechanisms of Disease: a 'DAMP' view of inflammatory arthritis. *Nat Clin Pract Rheum*, 3*,* 382-390 (2007)

9. J.O'Neill L.A..: When Signaling Pathways Collide: Positive and Negative Regulation of Toll-like Receptor Signal Transduction. *Immunity*, 29,12-20 (2008)

10. Aravalli R., P. Peterson & J.Lokensgard: Toll-like Receptors in Defense and Damage of the Central Nervous System. *J Neuroimm Pharm*, 2, 297-312 (2007)

11. Lotze M.T. & K.J. Tracey: High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol*, 5, 331-342 (2005)

12. Foell D., H. Wittkowski, T. Vogl & J.Roth: S100 proteins expressed in phagocytes: a novel group of damage-associated molecular pattern molecules. *J Leuk Biol* , 81, 28-37 (2007)

13. Creagh E.M. & L.A.J. O'Neill: TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity. *Trends Immunol*, 27, 352-357 (2006)

14. Janeway C.A., Jr. & R. Medzhitov: Innate immune recognition. *Annu Rev Immunol*, 20*.* 197-216 (2002)

15. Leifer C.A., M.N. Kennedy, A. Mazzoni, C. Lee, M.J. Kruhlak & D.M.Segal: TLR9 Is Localized in the Endoplasmic Reticulum Prior to Stimulation. *J Immunol*, 173, 1179-1183 (2004)

16. Uematsu S. & S. Akira: Toll-like receptors and innate immunity. *J Molec Med*, 84, 712-725 (2006)

17. Ishii K.J., C. Coban & S.Akira: Manifold Mechanisms of Toll-Like Receptor-Ligand Recognition. *J Clin Immunol*, 25, 511-521 (2005)

18. Bell J.K., G.E.D. Mullen, C.A. Leifer, A. Mazzoni, D.R. Davies & D.M.Segal: Leucine-rich repeats and pathogen recognition in Toll-like receptors. *Trends Immunol*, 24, 528-533 (2003)

19. Honda K., Y. Ohba, H. Yanai, H. Negishi, T. Mizutani, A. Takaoka, C. Taya & T. Taniguchi: Spatiotemporal regulation of MyD88-IRF-7 signalling for robust type-I interferon induction. *Nature*, 434, 1035-1040 (2005)

20. Fitzgerald K.A. & Z.J.Chen: Sorting out Toll Signals. *Cell*, 125, 834-836 (2006)

21. Klinman D.M., D. Verthelyi, F. Takeshita & K.J.Ishii: Immune recognition of foreign DNA: a cure for bioterrorism? *Immunity*, 11, 123-129 (1999)

22. Verthelyi D. & D.M. Klinman: Immunoregulatory activity of CpG oligonucleotides in humans and nonhuman primates. *Clin Immunol*, 109,, 64-71 (2003)

23. Rivest S., S. Lacroix, L. Vallieres, S. Nadeau, J. Zhang & N. Laflamme: How the Blood Talks to the Brain Parenchyma and the Paraventricular Nucleus of the Hypothalamus During Systemic Inflammatory and Infectious Stimuli. *Proc Soc Exp Biol Med*, 223, 22-38 (2000)

24. Lafon M., F. Megret, M. Lafage & C.Prehaud: The innate immune facet of brain. *J Molec Neurosci*, 29, 185-194 (2006)

25. Ma Y., J. Li, I. Chiu, Y. Wang, J.A. Sloane, J. Lu, B. Kosaras, R.L. Sidman, J.J. Volpe & T. Vartanian: Toll-like

receptor 8 functions as a negative regulator of neurite outgrowth and inducer of neuronal apoptosis. *J Cell Biol*, 175, 209-215 (2006)

26. Laflamme N. & S. Rivest: Toll-like receptor 4: the missing link of the cerebral innate immune response triggered by circulating gram-negative bacterial cell wall components. *FASEB*, 15, 155-163 (2001)

27. Olson J.K. & S.D. Miller: Microglia Initiate Central Nervous System Innate and Adaptive Immune Responses through Multiple TLRs. *J Immunol*, 173, 3916-3924 (2004)

28. Tonelli L.H., C.A. Gunsolly, E. Belyavskaya, A. R.Atwood & E.M. Sternberg: Increased pro-thyrotropin-releasing hormone transcription in hypophysiotropic neurons of Lewis rats. *J Neuroimmunol*, 153, 143-149 (2004)

29. Jack C.S., N. Arbour, J. Manusow, V. Montgrain, M. Blain, E. McCrea, A. Shapiro & J.P. Antel: TLR Signaling Tailors Innate Immune Responses in Human Microglia and Astrocytes. *J Immunol*, 175, 4320-4330 (2005)

30. Farina C., M. Krumbholz, T. Giese, G. Hartmann, F. Aloisi & E. Meinl: Preferential expression and function of Toll-like receptor 3 in human astrocytes. *J Neuroimmunol*, 159, 12-19 (2005)

31. Bsibsi M., R. Ravid, D. Gveric & J.M. vanNoort. Broad expression of Toll-like receptors in the human central nervous system. *J Neuropathol Exp Neurol*, 61, 1013-1021 (2002)

32. Ma Y., R.L. Haynes, R.L. Sidman & T.Vartanian: TLR8: An innate immune receptor in brain, neurons and axons. *Cell Cycle*, 6, 2859-2868 (2007)

33. Marsh B.J., R.L. Williams-Karnesky, & M.P.Stenzel-Poore: Toll-like receptor signaling in endogenous neuroprotection and stroke. *Neuroscience*, Aug 12 (2008)

34. Cameron J.S., L. Alexopoulou, J.A. Sloane, A.B. DiBernardo, Y. Ma, B. Kosaras, R. Flavell, S.M. Strittmatter, J. Volpe, R. Sidman & T. Vartanian: Toll-Like Receptor 3 Is a Potent Negative Regulator of Axonal Growth in Mammals. *J Neurosc.*, 27, 13033-13041 (2007)

35. Chen K., J. Huang, W. Gong, P. Iribarren, N.M. Dunlop & J.M.Wang: Toll-like receptors in inflammation, infection and cancer. *Int Immunopharmacol*, 7, 1271-1285 (2007)

36. Farina C., F. Aloisi & E.Meinl: Astrocytes are active players in cerebral innate immunity. *Trends Immunol*, 28, 138-145 (2007)

37. Carpentier P.A., W.S. Begolka, J.K. Olson, A. Elhofy, W. Karpus & S.D.Miller: Differential activation of astrocytes by innate and adaptive immune stimuli. *Glia*, 49, 360-374 (2005)

38. Zhao Y., M.A Rivieccio, S. Lutz, E. Scemes & C.F. Brosnan: The TLR3 ligand polyI: C downregulates connexin 43 expression and function in astrocytes by a mechanism involving the NF-kappaB and PI3 kinase pathways. *Glia*, 54, 775-785 (2006)

39. Tanaka T., K. Oh-hashi, H. Shitara, Y. Hirata & K.Kiuchi: NF-[kappa]B independent signaling pathway is responsible for LPS-induced GDNF gene expression in primary rat glial cultures. *Neurosci Let*, 431, 262-267 (2008)

40. Hanisch U.K., T. Johnson & J.Kipnis: Toll-like receptors: roles in neuroprotection? *Trends Neurosci*, 31, 176-182 (2008)

41. Wagner I., S. Sethi, W. Xiang, A. Giese, A. Ebner & H. Kretzschmar: Repeated Peripheral Administrations of CpG Oligodeoxynucleotides Lead to Sustained CNS Immune Activation. *Immunopharmacol Immunotoxicol*, 29, 413-424 (2008)

42. Deng G.M., Z.Q. Liu & A.Tarkowski: Intracisternally Localized Bacterial DNA Containing CpG Motifs Induces Meningitis. *J Immunol*, 167, 4616-4626 (2001)

43. Nadeau S. & S. Rivest: Role of Microglial-Derived Tumor Necrosis Factor in Mediating CD14 Transcription and Nuclear Factor kappa B Activity in the Brain during Endotoxemia. *J Neurosc.*, 20, 3456-3468 (2000)

44. Meyer T. & E. Stockfletch. Clinical applications of Toll-like receptor agonists. *Expert Opin Investig Drugs*, 17, 1051-1061 (2008)

45. Klinman D.M.: Immunotherapeutic uses of CpG oligodeoxynucleotides. *Nat Rev Immunol*, 4, 249-258 (2004)

46. Marsh B.J. & M.P.Stenzel-Poore: Toll-like receptors: novel pharmacological targets for the treatment of neurological diseases. *Curr Op Pharmacol* 8, 8-13 (2008)

47. Klinman D.M.: Use of CpG oligodeoxynucleotides as immunoprotective agents. *Expert Opin Bio. Ther*, 4, 937-946 (2004)

48. Flynn B., V. Wang, D.L. Sacks, R.A. Seder & D. Verthelyi: Prevention and Treatment of Cutaneous Leishmaniasis in Primates by Using Synthetic Type D/A Oligodeoxynucleotides Expressing CpG Motifs. *Infect Immun*, 73, 4948-4954 (2005)

49. Verthelyi D., R.T. Kenney, R.A. Seder, A.A. Gam, B. Friedag & D.M.Klinman: CpG oligodeoxynucleotides as vaccine adjuvants in primates. *J Immunol*, 168, 1659-1663 (2002)

50. Verthelyi D., M. Gursel, R.T. Kenney, J.D. Lifson, L.,Shuying, J.Mican, & D.M. Klinman: CpG Oligodeoxynucleotides protect normal and SIV infected macaques from Leishmania infection. *J Immunol*, 170, 4717-4723 (2003)

51. Puig M., A. Grajkowski, M. Boczkowska, C. Ausin, S.L. Beaucage, & D.Verthelyi: Use of thermolytic

protective groups to prevent G-tetrad formation in CpG ODN type D: structural studies and immunomodulatory activity in primates. *Nuc Acids Res*, 34 ,6488-6495 (2006)

52. Verthelyi D. & M.Puig: Structure/function of IFNa inducing CpG ODN. In K.J.Ishii and S.Akira (eds.), Nucleic Acids in Innate Immunity, CRC Press, Boca Raton, FL, 2008, PP: 113-128.

53. Bowman C.C., A. Rasley, S.L. Tranguch, & I.Marriott: Cultured astrocytes express toll-like receptors for bacterial products. *Glia*, 43, 281-291 (2003)

54. Dalpke A.H., M.K. Schafer, M. Frey, S. Zimmermann, J. Tebbe, E. Weihe &K.Heeg: Immunostimulatory CpG-DNA activates murine microglia. *J Immunol*, 168, 4854-4863 (2002)

55. Takeshita S., F. Takeshita, D.E. Haddad, N. Janabi & D.M. Klinman: Activation of microglia and astrocytes by CpG oligodeoxynucleotides. *Neuroreport*, 12, 1-4 (2001)

56. Mishra B., P. Mishra & J. Teale: Expression and distribution of Toll-like receptors in the brain during murine neurocysticercosis. *J Neuroimmunol*, 181, 46-56 (2006)

57. McKimmie C.S., N. Johnson, A.R. Fooks & J.K. Fazakerley: Viruses selectively upregulate Toll-like receptors in the central nervous system. *Biochem Biophys Res Com*, 336, 925-933 (2005)

58. Jackson A.C., J.P. Rossiter & M. Lafon: Expression of Toll-like receptor 3 in the human cerebellar cortex in rabies, herpes simplex encephalitis, and other neurological diseases. *J Neurovirol* 12, 229-234 (2006)

59. Klein M., B. Obermaier, B. Angele, H.W. Pfister, H. Wagner, U. Koedel & C.J. Kirschning: Innate Immunity to Pneumococcal Infection of the Central Nervous System Depends on Toll-Like Receptor (TLR) 2 and TLR4. *J Infec Dis*, 198,1028-1036 (2008)

60. Spinner D.S., I.S. Cho, S.Y. Park, J.I. Kim, H.C. Meeker, X. Ye, G. LaFauci, D.J. Kerr, M.J. Flory, B.S. Kim, R.B. Kascsak, T. Wisniewski, W.R. Levis, G.B. Schuller-Levis, R.I. Carp, E. Park & R.J.Kascsak: Accelerated prion disease pathogenesis in Toll-like receptor 4 (TLR4) signaling-mutant mice. *J Virol* (2008)

61. Mansur D.S., E.G. Kroon, M.L. Nogueira, R.M.E. Arantes, S.C.O. Rodrigues, S. Akira, R.T. Gazzinelli & M.A. Campos. Lethal Encephalitis in Myeloid Differentiation Factor 88-Deficient Mice Infected with Herpes Simplex Virus 1. *Am J Patho.*, 166, 1419-1426 (2005)

62. Daffis S., M.A. Samuel, M.S. Suthar, B.C. Keller, M. Gale, Jr. & M.S.Diamond: Interferon Regulatory Factor IRF-7 Induces the Antiviral Alpha Interferon Response and Protects against Lethal West Nile Virus Infection. *J Virol*, 82, 8465-8475 (2008)

63. Wang T., T. Town, L. Alexopoulou, J.F. Anderson, E. Fikrig & R.A. Flavell: Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nat Med*, 10, 1366-1373 (2004)

64. Lafon M., F. Megret, S.G. Meuth, O. Simon, M.L. Velandia Romero, M. Lafage, L. Chen, L. Alexopoulou, R.A. Flavell, C. Prehaud & H.Wiendl: Detrimental Contribution of the Immuno-Inhibitor B7-H1 to Rabies Virus Encephalitis. *J Immunol*, 180, 7506-7515 (2008)

65. Lepenies B., J. Cramer, G. Burchard, H. Wagner, C. Kirschning & T. Jacobs: Induction of experimental cerebral malaria is independent of TLR2/4/9. *Med Microb Immunol*, 197, 39-44 (2008)

66. Toqbe D., L. Schofield, G. Grau, B. Schneyder, V. Boissay, S. Charron, S. Rose, B. Beutler, V.F. Quesniaux & B.Ryffel: Murine cerebral malaria development is independent of toll-like receptor signaling. *Am J Patho.*, 170, 1640-1648 (2007)

67. Coban C., K.J. Ishii, S. Uematsu, N. Arisue, S. Sato, M. Yamamoto, T. Kawai, O. Takeuchi, H. Hisaeda, T. Horii & S.Akira: Pathological role of Toll-like receptor signaling in cerebral malaria. *Int Immunol*, 19, 67-79 (2007)

68. Pedras-Vasconcelos J.A., D. Goucher, M. Puig, L.H. Tonelli, V. Wang, S. Ito & D. Verthelyi: CpG Oligodeoxynucleotides Protect Newborn Mice from a Lethal Challenge with the Neurotropic Tacaribe Arenavirus. *J Immunol*, 176, 4940-4949 (2006)

69. Pedras-Vasconcelos J.A., M. Puig, C. Sauder, C. Wolbert, M. Ovanesov, D. Goucher & D. Verthelyi: Immunotherapy with CpG Oligonucleotides and Antibodies to TNF-{alpha} Rescues Neonatal Mice from Lethal Arenavirus-Induced Meningoencephalitis. *J Immunol*, 180, 8231-8240 (2008)

70. Ayerra de Holstein B.L., A.R. Teyssie, L.N. Knecher, S.R. Samoilovish, C.E. Coto & M. Weissebacher: Induccion de Interferon por el virus Tacaribe. *Medicina*, 41, 177-181 (1981)

71. Borden E.C. & N. Nathanson. Tacaribe virus infection of the mouse: an immunopathologic disease model. *Lab Invest*, 30, 465-473 (1974)

72. Parodi A.S., G.A. Schmunis & M. Weissebacher: Infection with viruses of the Tacaribe group in thymectomized mice. *Experientia*, 26, 665-666 (1970)

73. Boivin N., Y. Sergerie, S. Rivest & G. Boivin: Effect of Pretreatment with Toll-like Receptor Agonists in a Mouse Model of Herpes Simplex Virus Type 1 Encephalitis. *J Infec Dis*, 198, 664-672 (2008)

74. Verthelyi D. & R.A. Zeuner: Differential signaling by CpG DNA in DCs and B cells: not just TLR9. *Trends Immunol ,* 10, 519-522 (2003)

75. Verthelyi D.: Adjuvant properties of CpG ODN in primates. In W.M.Saltzman, H.Shen, and J.L.Brandsma (eds.), DNA vaccines: Methods and Protocols, Humana Press, Totowa, New Jersey, 2006, PP: 139-158.

76. Grajkowski A., J. Pedras-Vasconcelos, V. Wang, C. Ausin, S. Hess, D. Verthelyi & S.L. Beaucage: Thermolytic CpG-containing DNA oligonucleotides as potential immunotherapeutic prodrugs. *Nuc Acids Res*, 33, 3550-3560 (2005)

77. Vargas M.E. & B.A.Barres: Why Is Wallerian Degeneration in the CNS So Slow? *Ann Rev Neurosc*, 30, 153-179 (2007)

78. Schwartz M. & J. Kipnis: Model of Acute Injury to Study Neuroprotection. In Neuroprotection Methods and Protocols, 2007, PP: 41-53.

79. Tang S.C., T.V. Arumugam, X. Xu, A. Cheng, M.R. Mughal, D.G. Jo, J.D. Lathia, D.A. Siler, S. Chigurupati, X. Ouyang, T. Magnus, S. Camandola, & M.P. Mattson: Pivotal role for neuronal Toll-like receptors in ischemic brain injury and functional deficits. *Proc Natl Acad Sci*, 104, 13798-13803 (2007)

80. Lehnardt S., S. Lehmann, D. Kaul, K. Tschimmel, O. Hoffmann, S. Cho, C. Krueger, R. Nitsch, A. Meisel & J.R.Weber. Toll-like receptor 2 mediates CNS injury in focal cerebral ischemia. *J. Neuroimmuno.*, 190, 28-33 (2007)

81. Faulkner J.R., J.E. Herrmann, M.J. Woo, K.E. Tansey, N.B. Doan & M.V. Sofroniew: Reactive Astrocytes Protect Tissue and Preserve Function after Spinal Cord Injury. *J. Neurosci.*, 24, 2143-2155 (2004)

82. Bush T.G., N. Puvanachandra, C.H. Horner, A. Polito, T. Ostenfeld, C.N. Svendsen, L. Mucke, M.H. Johnson & M.V. Sofroniew: Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. *Neuron*, 23: 297-308 (1999)

83. Crutcher K., H. Gendelman, J. Kipnis, J. Perez-Polo, V. Perry, P. Popovich & L. Weaver: Debate: Is Increasing Neuroinflammation Beneficial for Neural Repair?. *J Neuroimmune Pharmacol*, 1, 195-211 (2006)

84. Kipnis J. & M. Schwartz. Controlled autoimmunity in CNS maintenance and repair. *Neuro Molec Med*, 7, 197-206 (2005)

85. Johnson T.V., C.B. Camras & J. Kipnis: Bacterial DNA Confers Neuroprotection after Optic Nerve Injury by Suppressing CD4+CD25+ Regulatory T-Cell Activity. *Invest Ophthalmo. Vis Sc.*, 48, 3441-3449 (2007)

86. Kigerl K., W. Lai, S. Rivest, R. Hart, A.R. Satoskart & P.G. Popovich: Toll-like receptor (TLR)-2 and TLR-4 regulate inflammation, gliosis, and myelin sparing after spinal cord injury. *J Neurochem*, 102, 37-50 (2007)

87. Tasaki K., C.A. Ruetzler, T. Ohtsuki, D. Martin, H. Nawashiro & J.M.Hallenbeck: Lipopolysaccharide pre-treatment induces resistance against subsequent focal cerebral ischemic damage in spontaneously hypertensive rats. *Brain Resh*, 748, 267-270 (1997)

88. Rosenzweig H.L., M. Minami, N.S. Lessov, S.C. Coste, S.L. Stevens, D.C. Henshall, R. Meller, R.P. Simon & M.P.Stenzel-Poore: Endotoxin preconditioning protects against the cytotoxic effects of TNF[alpha] after stroke: a novel role for TNF[alpha] in LPS-ischemic tolerance. *J Cereb Blood Flow Metab*, 27, 1663-1674 (2007)

89. Stevens S.L., T.M.P. Ciesielski, B.J. Marsh, T. Yang, D.S. Homen, J.L. Boule, N.S. Lessov, R.P. Simon & M.P. Stenzel-Poore: Toll-like receptor 9: a new target of ischemic preconditioning in the brain. *J Cereb Blood Flow Metab*, 28, 1040-1047 (2008)

90. Veldhuis W.B., J.W. Derksen, S. Floris, P.H. van der Meide, H.E. de Vries, J. Schepers, I.M.P. Vos, C.D. Dijkstra, L.J. Kappelle, K. Nicolay & P.R. Bar: Interferon-Beta Blocks Infiltration of Inflammatory Cells and Reduces Infarct Volume After Ischemic Stroke in the Rat. *J Cereb Blood Flow Metab*, 23, 1029-1039 (2003)

91. Veldhuis W.B., S. Floris, P.H. van der Meide, I.M.P. Vos, H.E. de Vries, C.D. Dijkstra, P.R. Bar & K.Nicolay: Interferon-Beta Prevents Cytokine-Induced Neutrophil Infiltration and Attenuates Blood-Brain Barrier Disruption. *J Cereb Blood Flow Metab*, 23, 1060-1069 (2003)

92. Tahara K., H.D. Kim, J.J. Jin, J.A. Maxwell, L. Li & K.I. Fukuchi. Role of toll-like receptor signalling in A{beta} uptake and clearance. *Brain*, 129, 3006-3019 (2006)

93. Wirths O., H. Breyhan, A. Marcello, M.C. Cotel, W. Brnck & T.A. Bayer: Inflammatory changes are tightly associated with neurodegeneration in the brain and spinal cord of the APP/PS1KI mouse model of Alzheimer's disease. *Neurobiol Aging*, July 25 (2008)

94. Tang S.C., J.D. Lathia, P.K. Selvaraj, D.G. Jo, M.R. Mughal, A. Cheng, D.A. Siler, W.R. Markesbery, T.V. Arumugam & M.P. Mattson: Toll-like receptor-4 mediates neuronal apoptosis induced by amyloid [beta]-peptide and the membrane lipid peroxidation product 4-hydroxynonenal. *Exper Neurol*, 213, 114-121 (2008)

95. Won E., M.C. Zahner, E. Grant, P. Gore & M.R. Chicoine: Analysis of antitumoral mechanisms of lipopolyshacharide against glioblastoma multiforme. *Anti-Cancer Drugs*, 14, 457-466 (2003)

96. Chicoine M.R., M. Zahner, E. Won, R. Kalra, T. Kitamura, A. Perry, & R. Higashikubo: The *in vivo* antitumoral effects of lipopolysaccharide against glioblastoma multiforme are mediated in part by Toll-like receptor 4. *Neurosurgery*, 60, 372-380 (2007)

97. Prins R.M., N. Craft, K.W. Bruhn, H. Khan-Farooqi, R.C. Koya, R. Stripecke, J.F. Miller & L.M.Liau: The

TLR-7 Agonist, Imiquimod, Enhances Dendritic Cell Survival and Promotes Tumor Antigen-Specific T Cell Priming: Relation to Central Nervous System Antitumor Immunity. *J Immunol*, 176, 157-164 (2006)

98. Carpentier A.F., L. Chen, F. Maltonti & J.Y.Delattre: Oligodeoxynucleotides containing CpG motifs can induce rejection of a neuroblastoma in mice. *Cancer Res*, 59. 5429-5432 (1999)

99. Meng Y., M. Kujas, Y. Marie, S. Paris, J. Thillet, J.Y. Delattre & A.F.Carpentier: Expression of TLR9 within human glioblastoma. *J Neurooncol*, 88, 19-25 (2008)

100. Swann J.B., M.D. Vesely, A. Silva, J. Sharkey, S. Akira, R.D. Schreiber & M.J. Smyth: Demonstration of inflammation-induced cancer and cancer immunoediting during primary tumorigenesis. *Proc Natl Acad Sci USA*, 105, 652-656 (2008)

101. Rakoff-Nahoum S. & R. Medzhitov: Regulation of Spontaneous Intestinal Tumorigenesis Through the Adaptor Protein MyD88. *Science*, 317, 124-127 (2007)

102. Marshak-Rothstein A. & I.R. Rifkin: Immunologically Active Autoantigens: The Role of Toll-Like Receptors in the Development of Chronic Inflammatory Disease. *Annu Rev Immunol*, 25, 419-441 (2007)

103. Zeuner R.A., K.J. Ishii, M.J. Lizak, I. Gursel, H. Yamada, D.M. Klinman & D. Verthelyi: Reduction of CpG-induced arthritis by suppressive oligodeoxynucleotides. *Arthritis Rheum*, 46, 2219-2224 (2002)

104. Barrat F.J. & R.L.Coffman: Development of TLR inhibitors for the treatment of autoimmune diseases. *Immunol Rev*, 223, 271-283 (2008)

105. Prinz M., F. Garbe, H. Schmidt, A. Mildner, I. Gutcher, K. Wolter, M. Piesche, R. Schroers, E. Weiss, C. Kirschning, B. Rochford, W. Bruck & B. Becher: Innate immunity mediated by TLR9 modulates pathogenicity in an animal model of multiple sclerosis. *J Clin Invest*, 116, 456-464 (2006)

106. Marta M., A. Andersson, M. Isaksson, O. Kampe & A. Lobell: Unexpected regulatory roles of TLR4 and TLR9 in experimental autoimmune encephalomyelitis. *Eur J Immunol*, 38, 565-575 (2008)

107. Visser L., H. Jan de Heer, L.A. Boven, D. van Riel, M. van Meurs, M.J. Melief, U. Zahringer, J. van Strijp, B.N. Lambrecht, E.E. Nieuwenhuis & J.D.Laman; Proinflammatory Bacterial Peptidoglycan as a Cofactor for the Development of Central Nervous System Autoimmune Disease. *J Immunol*, 174, 808-816 (2005)

108. Segal B.M., D.M. Klinman & E.M.Shevach: Microbial products induce autoimmune disease by an IL-12 dependent process. *J Immunol*, 158, 5087-5091 (1997)

109. Segal B.M., J.T. Chang & E.M.Shevach: CpG oligonucleotides are potent adjuvants for the activation of autoreactive encephalotogenic T cells *in vivo*. *J Immunol*, 164, 5683-5688 (2000)

110. Ponomarev E.D., L.P. Shriver, K. Maresz, J. Pedras-Vasconcelos, D. Verthelyi & B.N. Dittel: GM-CSF Production by Autoreactive T Cells Is Required for the Activation of Microglial Cells and the Onset of Experimental Autoimmune Encephalomyelitis. *J Immunol*, 178, 39-48 (2007)

111. Glezer I., A.R. Simard & S.Rivest: Neuroprotective role of the innate immune system by microglia. *Neuroscience*, *147*, 867-883 (2007)

112. Touil T., D. Fitzgerald, G.X. Zhang, A. Rostami & B. Gran: Cutting Edge: TLR3 Stimulation Suppresses Experimental Autoimmune Encephalomyelitis by Inducing Endogenous IFN-beta. *J Immunol*, 177, 7505-7509 (2006)

113. Guo B., E.Y. Chang & G. Cheng: The type I IFN induction pathway constrains Th17-mediated autoimmune inflammation in mice. *J Clin Invest*, 118, 1680-1690 (2008)

114. Prinz M., H. Schmidt, A. Mildner, K.P. Knobeloch, U.K. Hanisch, J. Raasch, D. Merkler, C. Detje, I. Gutcher, J.R. Mages, R. Lang, R. Martin, R. Gold, B. Becher, W. Brnck, & U. Kalinke: Distinct and Nonredundant *In vivo* Functions of IFNAR on Myeloid Cells Limit Autoimmunity in the Central Nervous System. *Immunity*, 28, 675-686 (2008)

115. Verthelyi D. & D.M. Klinman: CpG ODN: Safety considerations. In E.Raz (ed.), Microbial DNA and immune modulation., Humana Press, CA, 2002, PP: 385-396.

116. Evans J.T., C.W. Cluff, D.A. Johnson, M.J. Lacy, D.H. Persing & J.R.Baldridge: Enhancement of antigen-specific immunity via the TLR4 ligands MPL adjuvant and Ribi.529. *Exp Rev Vaccines*, 2, 219-229 (2003)

117. Krieg A.M.: CpG motifs in bacterial DNA and their immune effects. *Annu Rev Immunol*, 20, 709-760 (2002)

118. Krieg A.M.: Therapeutic potential of Toll-like receptor 9 activation. *Nat Rev Drug Discov*, 5, 471-484 (2006)

119. Casella C. & T. Mitchell: Putting endotoxin to work for us: Monophosphoryl lipid A as a safe and effective vaccine adjuvant. *Cel Molec Life Sc,i* 65, 3231-3240.

120. Carpentier A., F. Laigle-Donadey, S. Zohar, L. Cepelle, A. Behin, A. Tibi, N. Martin-Duverneuil, M. Sanson, L. Lacomblez, S. Taillibert, L. Puybasset, R. Van Effenterre, J.Y. Delattre & A.F. Carpentier: Phase 1 trial of a CpG oligodeoxynucleotide for patients with recurrent glioblastoma. *Neuro Oncol*, 8, 60-66 (2006)

121. de la Torre J.C.: Pathophysiology of Neuronal Energy Crisis in Alzheimer's Disease. *Neurodeg Dis*, 5, 126-132 (2008)

122. Skaper S.: The Brain as a Target for Inflammatory Processes and Neuroprotective Strategies. *NY Acad Sci*, 1122, 23-34 (2007)

123. Arshavsky Y.I.: Alzheimer's disease, brain immune privilege and memory: a hypothesis. *J Neural Trans*, 113, 1697-1707 (2006)

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