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It's all in the family: multiple Toll-like receptors offer promise as novel therapeutic targets for stroke neuroprotection

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Abstract

Ischemic tolerance is a biological process that can be utilized to unlock the brain's own endogenous protection mechanisms and, as such, holds true promise for patients at risk of ischemic injury. Experimentally, preconditioning with various Toll-like receptor (TLR) agonists has now been demonstrated to successfully attenuate ischemic damage, partly through genomic reprogramming of the body's response to stroke. This treatment diminishes the inflammatory response to stroke and at the same time enhances the production of anti-inflammatory cytokines and neuroprotective mediators. This review discusses recent discoveries about the role of TLRs in preconditioning and ischemic tolerance.

Keywords

ischemic tolerance; preconditioning; stroke; Toll-like receptors

Ischemia-reperfusion injury induces neuronal damage by disrupting the delicate electrical and chemical balance in the brain. Stroke also elicits an inflammatory response that contributes to neuronal damage through systemic immune activation and infiltration. Following stroke, leukocytes, macrophages and neutrophils migrate through the damaged BBB to enter the CNS. In addition to the activation of the peripheral immune system, stroke activates microglia, endothelial cells and astrocytes in the CNS. Attenuation of the deleterious effects of the inflammatory response can be a powerful means to improve stroke outcome. Delayed preconditioning with Toll-like receptor (TLR) agonists appears to reduce ischemic damage by reprogramming the inflammatory response to stroke.

Preconditioning is a phenomenon whereby exposure to a modest amount of an otherwise harmful stimulus can protect against a subsequent, more severe threat. The preconditioning stimulus lessens damage from ischemia-reperfusion injury, leading to ischemic tolerance (reviewed in [1]). Ischemic tolerance can be induced by numerous distinct stimuli including mild epilepsy, brief exposure to hypoxia, ischemia or hypothermia, spreading depression, hypoperfusion and moderate inflammatory activation. Preconditioning is referred to as rapid or delayed depending on whether tolerance to injury occurs immediately or requires substantial time to develop. Delayed preconditioning develops over hours or days and requires *de novo* protein synthesis. By contrast, rapid preconditioning takes place within minutes to hours and does not require protein synthesis [2].

Stroke is the leading cause of disability and the third leading cause of death in the USA yet there is only one treatment for stroke, tissue-plasminogen activator (t-PA) — a thrombolytic drug that can dissolve an embolus. Unfortunately, t-PA is only effective in a small percentage of patients. This is due to the very short time window during which this drug can be safely used because of the time and type of tests needed to diagnose the type of stroke in order to avoid treating hemorrhagic stroke patients with t-PA. Numerous promising treatments developed at the laboratory bench have failed to translate into effective clinical approaches; therefore, there remains a critical need for new innovative approaches. While many types of preconditioning stimuli are not reasonable treatments for patients, TLR ligands hold great potential for clinical use and have been used to treat other diseases. Preconditioning treatments could be a viable option in patients with a high risk of stroke, such as those who have had a transient ischemic attack or who are at risk of suffering ischemia during major surgery. This review will discuss the role of TLRs in preconditioning as novel neuroprotectants against ischemia.

TLR family

Toll-like receptors comprise a family of pattern-recognition receptors involved in innate immune system functions. Structurally, TLRs are characterized by extracellular leucine-rich repeat motifs and an intracellular Toll-interleukin 1 receptor (TIR) domain [3,4]. Functionally, TLRs are characterized by their ability to recognize common pathogen molecules or pathogen-associated molecular patterns, such as lipopolysaccharide (LPS) and flagellin.

Toll-like receptors can be broadly classified into six subfamilies based on amino acid homologies: TLR2, 3, 4, 5, 9 and 11. All TLRs are located on the cell surface except for TLR3 and those in the TLR9 family, which are located on endosomes. The TLR2 family consists of TLR1, 2 and 6. TLR2 is activated by components from a variety of microorganisms, including peptidoglycan in the bacterial cell wall and lipoteichoic acid from Gram-positive bacteria. Furthermore, TLR2 dimerizes with TLR1 and 6 to confer additional discrimination among microbial components. The TLR2/TLR6 dimer is activated by diacyl lipopeptides, whereas the TLR2/TLR1 dimer is involved in the recognition of subtle differences in different triacyl lipopeptides [5–9]. TLR3, which is in a family by itself, recognizes double-stranded RNA produced by various viruses, the endogenous ligand self mRNA, and the synthetic nucleoside moiety [10,11]. TLR5 recognizes flagellin — a predominant protein in flagellar that is responsible for motility in some bacteria [12]. TLR4 recognizes LPS from Gram-negative bacteria and a range of host-derived molecules, such as heat-shock proteins and various extracellular matrix components, including fibronectin, hyaluronic acid and heparan sulfate [13–15]. These endogenous host ligands are released in response to injury and inflammation, and are thought to be involved in tissue remodeling. The TLR9 subfamily includes TLR7, 8 and 9, which are activated by single-stranded RNA that is present in certain classes of RNA viruses. TLR7 can also be activated by a class of small synthetic compounds known as imidazoquinolines. TLR9 is activated by unmethylated CpG motifs that are found in bacterial

and viral DNA, synthetic oligodeoxynucleotides and endogenous DNA complexes released from dying cells [16–19]. Little is known about the recently identified TLR11 family that consists of TLRs 11–13, including their ligands. Early reports suggest that TLR11 responds to uropathogenic bacteria [20] and a profilin-like protein [21].

TLR distribution

Toll-like receptors are expressed on a wide variety of cell types throughout the body. Most notably, TLRs are located on immune system cells, such as macrophages, T cells, B cells and dendritic cells, where they play an important role in the detection of pathogens and the initiation of an immune response. These sentinel receptors have also been detected on diverse cells, including epithelial cells and smooth muscle cells [22,23]. With this broad distribution, TLRs are poised to respond to a cerebral ischemic challenge or to modulate cells affected by ischemic injury, especially since their expression has been found on CNS cells. While a definitive, systematic survey of TLR expression in the CNS has not yet been completed, published reports have shown TLR expression throughout the nervous system (extensively reviewed in [24]). In brief, expression of TLRs 1–9 has been reported on microglia [25,26], and TLRs 1–8 and 11–13 on neurons [27–33]. In addition, TLRs 1–7, 9, 10 and 13 are expressed on astrocytes, TLR2 and 3 on oligodendrocytes and endothelial cells have been demonstrated to express TLR3 and 13 [22,25,31,34–37]. It is important to note that cells with a different repertoire of TLRs will induce different downstream signals and cytokines in response to a danger signal. In addition, each cell type will have a unique response to a given TLR activation. Therefore, a comprehensive investigation of TLR activity in diverse cell types and how each cell type contributes to TLR preconditioning would be valuable to the understanding of TLR-mediated tolerance.

TLR pathways

Upon ligand binding, TLRs dimerize, undergo a conformational change and initiate signaling. Two signaling pathways used by TLRs have been identified: the MyD88 pathway and the TIR domain-containing adaptor inducing interferon (TRIF) pathway [38]. In brief, signaling down the MyD88 pathway leads to the activation of NF- κ B and the production of proinflammatory cytokines, whereas signaling through the TRIF pathway induces the production of interferons (IFNs) and other IFN-inducible genes. The MyD88 pathway is utilized by all TLRs except TLR3. Interestingly, TLR3 is unique in exclusively signaling through the TRIF pathway, while TLR4 is the only TLR that signals through both cascades (Figure 1).

MyD88 pathway

For most TLRs, MyD88 is recruited directly to the TLR itself. The exceptions to this rule are TLR2 and 4, which require the adaptor protein TIR domain-containing adaptor protein (also known as MyD88 adapter-like protein). MyD88 recruitment is followed by recruitment of the IL-1 receptor-associated kinase (IRAK)-4 to the receptor complex and phosphorylation of IRAK1. Activated IRAK1 binds the adaptor protein TNF receptor-associated factor (TRAF)-6, in turn causing the dissociation of the IRAK1—TRAF6 complex. The dissociated IRAK1—TRAF6 complex interacts with the TGF- β -activated kinase (TAK)-1 and two adaptor proteins, TGF- β -binding protein 1 and 2 or 3. The subsequent phosphorylation of the TAK1 complex induces TAK1 to activate the inhibitor of NF- κ B kinase complex, which then degrades inhibitor of NF- κ B to release NF- κ B, allowing the transcription factor to translocate to the nucleus, resulting in the production of proinflammatory cytokines, such as TNF- α and IL-6.

Activation of TLR7 and 9 also leads to the production of type I IFNs. This occurs through the binding of TRAF3 and 6, which leads to the activation and nuclear translocation of several

IFN-regulatory factors (IRF) (e.g., IRF1, 3, 5 and 7) and results in the transcription and production of the type I IFNs, IFN α and β , as well as expression of IFN-inducible genes.

TRIF pathway

Toll-like receptor 3 is unique among the TLRs as it signals exclusively through the TRIF pathway. TLR3 dimerization in response to ligand binding leads to recruitment of the adapter protein TRIF, TRAF3 and activation of TAK-binding kinase 1 and noncanonical I κ B kinases. Activation of IRF3 and 7 ensues, which leads to induction of Type I IFNs and IFN-inducible genes. TRIF can also activate TRAF6 directly or via receptor (TNF-receptor superfamily)-interacting serine—threonine protein kinase-1, leading to the activation of the IKK complex and, subsequently, NF- κ B.

Toll-like receptor 4 signals via the MyD88 and TRIF pathways. The TLR4—TRIF pathway is largely similar to that described for TLR3; however, TLR4 associates directly with TRIF-related adapter molecule and, in turn, indirectly with TRIF. Subsequent induction of IFN β and IFN-inducible genes occurs through the transcription factors IRF1, 3 and 5.

Preconditioning with TLRs induces neuroprotection against ischemia

As mentioned previously, ischemia induces a profound inflammatory response that contributes significantly to damage. Certain molecules associated with injury, such as heat-shock proteins and extracellular matrix components, may act as endogenous ligands to stimulate TLRs, thereby modulating the inflammatory response. In fact, TLR2 and 4 have both been demonstrated to directly contribute to ischemic injury in the brain [33,39,40]: TLR2-knockout mice exhibit less damage than wild-type mice and mice lacking functional TLR4 have smaller infarcts than their wild-type counterparts. Analogous to the endogenous role of TLR in brain ischemia, TLR2 and 4 contribute to ischemic injury in the kidney [41], and TLR4 contributes to ischemic injury in the liver [42]. By contrast, TLR2 was demonstrated to have a protective effect for ischemia in the small bowel of the mouse [43]. Since the role of TLRs in various ischemia-reperfusion injury models has been extensively reviewed [44], this review focuses on the phenomenon of inducing neuroprotection against cerebral ischemia through preconditioning with TLRs.

Paradoxically, although stimulation of TLRs during ischemia may contribute to ischemic injury, activation of certain TLRs prior to ischemia provides robust protection in a variety of organs. LPS pretreatment decreases damage from ischemia-reperfusion injury in the rat heart [45]. A recent report demonstrated that LPS preconditioning could protect the rat retina from ischemic damage [46]. In addition, TLR4 was shown to have neuroprotective effects when stimulated prior to ischemia stroke [47]. A low systemic dose of LPS given to spontaneously hypertensive rats attenuated brain damage incurred by a middle cerebral artery occlusion (MCAO). Subsequent studies demonstrated that neuroprotection induced by LPS preconditioning occurs within 1 day of exposure and lasts approximately 1 week [48,49]. The observed tolerance to ischemia depends on *de novo* protein synthesis and involves new gene expression [50].

Three other TLR family members (TLR2, 7 and 9) have been shown to induce neuroprotection against an ischemic injury when administered prior to MCAO. Preconditioning with CpG1826 (a TLR9 ligand), ameliorated damage incurred by MCAO in a dose- and time-dependent manner [51]. Similarly, when TLR2 was stimulated with its ligand Pam3CSK4 24 h prior to MCAO, BBB function was preserved with a reduced loss of the tight junction protein occludin [52]. Brain edema was also lessened with Pam3CSK4 pretreatment and mice showed significantly smaller infarcts. Studies from our laboratory demonstrated that TLR7 activation leads to profound neuroprotection in response to transient ischemia [Leung P, Yang T, Simon

R, Stenzel Poore M, Unpublished Data]. In addition to systemic actions, TLR ligands may also protect CNS cells directly, as preconditioning with LPS, CpG and TLR7 ligands 24 h prior to oxygen—glucose deprivation in an *in vitro* model of ischemia, reduced cell death in primary mouse cortical cultures [49,51]. Activation of TLR3 signals exclusively through the TRIF pathway, eliciting a unique transcription profile characterized by activity of IRF3 and 7 transcription factors and leading to robust production of IFN β and IFN-inducible genes [53, 54]. This common IFN response to TLRs 4, 7 and 9 suggests that TLR3 may also be a viable preconditioning target. Indeed, other reports have demonstrated neuroprotection following TLR3 stimulation in the context of a mouse model of multiple sclerosis [55] and general neuronal survival [35]. Owing to its unique signaling profile and broad expression in the CNS, investigation of TLR3 in the context of preconditioning and stroke may offer insights into the mechanism of TLR preconditioning in general. In the future, we may discover that preconditioning with any TLR ligand confers protection against subsequent ischemic insult; however, owing to the diversity among TLRs in regards to location and diverse cellular responses, it is highly probable that there will be different mechanisms behind the preconditioning neuroprotection.

Site of TLR neuroprotection

The site of action for TLR-induced neuroprotection is not yet clear. We can identify three potential sites of action for TLR-mediated ischemic tolerance: peripheral cells, brain endothelial cells and CNS cells. TLR agonists administered peripherally induce protection in the brain, suggesting that the agonists may act on peripheral cells whose responses then indirectly lead to the preservation of brain tissue. With preconditioning, TLR agonists administered peripherally induce the production of cytokines, and LPS preconditioning reduced the number of activated monocytes and neutrophils [48], which may result in reduced infiltration into the brain following ischemia by these inflammatory cells. CpG preconditioning also induces a natural killer cell-associated peripheral response to stroke that may promote the production of IFN [56].

Alternatively, TLR agonists may pass into the brain to act directly on the CNS cells to reprogram their response to ischemia. Our data from an *in vitro* model of ischemia (oxygen—glucose deprivation) indicate that TLR ligands can directly protect cells in mixed cortical cultures. A report using TLR4 bone marrow chimeric mice also demonstrate that peripheral TLR4 stimulation was not required as these mice, with no TLR4 in the periphery, still exhibited a strong cytokine response in the CNS [57]. Furthermore, microarray analyses indicated that in addition to the expected gene expression changes in the periphery, dramatic changes in gene expression were also seen in the brain following systemic administration of CpG and LPS [Marsh B, Lessov N, Stenzel-Poore M, Unpublished Data]. While this does not conclusively answer the question of whether TLR agonists cross the normal, undamaged BBB directly to effect changes in the brain during the preconditioning phase, it does suggest that TLR agonists do have the ability to exert reprogramming effects in brain cells following systemic administration no matter what the route. A third possibility is that TLR agonists act on brain endothelial cells since these cells make up the backbone of the BBB; thus, protecting them could lead to reduced BBB dysfunction and inflammatory-cell infiltration, in turn reducing ischemic damage. Further work is needed to elucidate the critical site of TLR action in ischemic tolerance.

Potential mechanisms of TLR-induced protection

Understanding the molecular mechanisms and genes responsible for neuroprotection against ischemia will provide insight into cell-survival mechanisms and guidance for future stroke treatment strategies. Our laboratory has conducted gene-profiling analyses to begin to define

the elements critical for TLR-induced neuroprotection [58]. Based on our studies, we postulate that three key phases to preconditioning and neuroprotection exist. The initiation phase is the direct response to the preconditioning stimulus, which is followed by a reprogramming phase wherein the response to injury is redirected, leading to an effector phase, which is characterized by the neuroprotective phenotype. The proinflammatory response that is initiated following treatment with a TLR ligand may be a critical component to the setup of neuroprotection. TNF- α has been identified as a key pro inflammatory cytokine involved in preconditioning. Preconditioning with LPS or CpG induces the production of the inflammatory cytokine TNF- α ; however, following MCAO, mice that have been preconditioned display reduced levels of TNF- α in the serum as well as diminished levels of cellular TNF-receptor (TNFR)-1 with increased levels of neutralizing soluble TNFR-1 [49]. Thus, preconditioning leads to a diminished inflammatory response after ischemia. The muted inflammatory response is also evidenced by a decrease in microglial activation and neutrophil infiltration into the brain as well as by reduced activation of monocytes in the periphery following MCAO [48]. TNF- α is required for LPS and CpG preconditioning since TNF- α -deficient mice cannot be protected by either LPS or CpG preconditioning [49,51]. TNF- α itself is an effective preconditioning stimulus when given systemically prior to insult [59]. Preconditioning also results in a suppression of NF- κ B activity following ischemic insult. Inhibition of NF- κ B action by the 5-lipoxygenase inhibitor also leads to protection from ischemia [60].

Coupled with the suppression of the TNF- α and proinflammatory response is an enhancement of the anti-inflammatory response. An IFN fingerprint was revealed in genomic microarray studies following ischemic insult after TLR4 or 9 preconditioning [56]. This IFN fingerprint comprises an increase in IRF-mediated transcription and increased production of type I IFNs in the brain. Further implicating an important role for an IFN system is the fact that IRF3-deficient mice are not protected by LPS preconditioning [Marsh B, Lessov N, Stenzel-Poore M, Unpublished Data]. This anti-inflammatory response may be a key element of the effector phase of preconditioning. Several lines of evidence emphasize the neuroprotective potential of IFNs. IFN β administered subcutaneously after stroke significantly reduces infarct volume [61,62]. This IFN treatment is associated with improved integrity of the BBB consisting of decreased MMP9, limited neutrophil or macrophage entry into the brain and reduced leakage of contrast agent in MRI studies [63,64]. In addition, IFN β given directly into the cerebral ventricle at the time of MCAO is neuroprotective. Thus, increased IFN β in the brain appears to be beneficial in the setting of ischemic injury.

How is the shift from the proinflammatory to anti-inflammatory response achieved? The key may be the redirection of TLR signaling pathways (Figure 1). Two adaptor proteins that signal downstream of the TLR4 receptor — MyD88 and TRIF — are candidates in determining the inflammatory outcome. MyD88-deficient mice can be preconditioned with LPS, suggesting that MyD88 is not crucial to TLR4 preconditioning. By contrast, TRIF-deficient mice were not protected by LPS preconditioning, suggesting that TRIF is the key adapter protein for this TLR4 response [Marsh B, Lessov N, Stenzel-Poore M, Unpublished Data]. TRIF stimulation leads to the activation of two diverse categories of transcription factors: NF- κ B for proinflammatory responses and IRFs for anti-inflammatory responses. Preconditioning may redirect TLR4— TRIF signaling from NF- κ B prior to ischemic insult to IRF-mediated protection following injury. Initial stimulation of TLR4 by LPS activates NF- κ B and the production of proinflammatory cytokines such as TNF- α . This initial moderate TLR4 stimulation reprograms the subsequent TLR4 response to ischemia through the activation of IRFs and IFN production.

Several pathway inhibitors may redirect the response to IRF activation. For example, the tripartite-motif protein TRIM30 α is upregulated after LPS and CpG preconditioning in both the blood and the brain and it is upregulated following ischemic insult in the brain [Stevens S,

Lessov N, Stenzel-Poore M, Unpublished Data]. *TRIM30α* acts to destabilize the TAK1 complex, thus blocking NF-κB activation. Tollip is another significant inhibitor that is upregulated after LPS preconditioning and following ischemia in the brain [Marsh B, Lessov N, Stenzel-Poore M, Unpublished Data]. Tollip also inhibits the NF-κB response. These inhibitors may serve to redirect signaling towards IRF activation after ischemia.

Conclusion & future perspective

Innovative and exciting data on TLRs and their roles in ischemic damage and ischemic tolerance have emerged. Gene expression studies have identified key effector pathways. In addition, these studies support the view that neuro protection involves fundamental genomic reprogramming of the response to ischemia, which redirects signaling away from cell death towards cell survival.

The use of TLR agonists to prevent ischemic injury offers real promise as a neuroprotective therapy, which involves treatment prior to an ischemic event. This approach could be useful to patients at high risk of ischemia, including those with a new transient ischemic attack (300,000/year in the USA) — 10% of whom will have a stroke within 90 days and 50% of those patients will suffer from a stroke within 48 h. Furthermore, approximately 50% of patients who undergo coronary artery bypass surgery suffer long-term cognitive decline from intraoperative ischemia — preoperative treatment of such patients (336,000 annually) may reduce ischemic injury and morbidity. In addition, individuals who have had a stroke are at high risk of recurrent stroke (25–40% within 5 years). Prophylactic neuroprotection of these high-risk populations has enormous potential to protect patients from devastating neurological complications and death.

Executive summary

Introduction

- Stroke is a leading cause of disability and death, and with only a single approved stroke treatment there is a desperate need for new approaches.
- Ischemic tolerance can be induced by preconditioning with a subinjurious amount of an otherwise harmful stimulus that can protect against a subsequent ischemic injury.
- Toll-like receptors (TLRs), a family of pattern-recognition receptors, are found on a wide variety of cell types throughout the body and are activated by both exogenous and endogenous molecules. These receptors may be potential therapeutic targets for ischemic tolerance.

Preconditioning with TLR ligands induces neuroprotection against ischemia

- Stimulation of TLRs leads to the production of proinflammatory cytokines, type I interferons (IFNs) and other anti-inflammatory cytokines.
- Paradoxically, although stimulation of TLRs during ischemia may contribute to ischemic injury, activation of certain TLRs prior to ischemia provides robust neuroprotection:
 - Activation of TLR2 and 4 at the time of ischemia exacerbates ischemic injury;
 - Preconditioning with TLRs 2, 4, 7 or 9 ligands protects the brain from ischemic damage.

Potential mechanisms of TLR-induced protection

- TNF α is required for the development of TLR4- and 9-mediated ischemic tolerance.
- Type I IFN appears to be beneficial in the setting of ischemic injury, and genomic microarray studies revealed a shared IFN fingerprint between TLR4 and 9 preconditioning.
- TLR4-mediated ischemic tolerance may be achieved by inhibition of the inflammatory signaling pathway and enhancement of the IFN and survival pathway.

Conclusions & future perspective

- Neuroprotection by TLRs involves fundamental genomic reprogramming of the response to ischemia, which redirects signaling away from cell death towards cell survival.
- The use of TLR agonists as a prophylactic treatment offers real promise as a neuroprotective therapy for high-risk patient populations.

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Bibliography

Papers of special note have been highlighted as: ■ of interest

1. Gidday JM. Cerebral preconditioning and ischaemic tolerance. *Nat. Rev. Neurosci* 2006;7:437–448. [PubMed: 16715053] Thorough overview of the preconditioning and tolerance paradigm.
2. Dirnagl U, Simon RP, Hallenbeck JM. Ischemic tolerance and endogenous neuroprotection. *Trends Neurosci* 2003;26:248–254. [PubMed: 12744841]
3. Bowie A, O'Neill LA. The interleukin-1 receptor/Toll-like receptor superfamily: signal generators for pro-inflammatory interleukins and microbial products. *J. Leukoc. Biol* 2000;67:508–514. [PubMed: 10770283]
4. Slack JL, Schooley K, Bonnert TP, et al. Identification of two major sites in the type I interleukin-1 receptor cytoplasmic region responsible for coupling to pro-inflammatory signaling pathways. *J. Biol. Chem* 2000;275:4670–4678. [PubMed: 10671496]
5. Chuang TH, Ulevitch RJ. Cloning and characterization of a sub-family of human Toll-like receptors: hTLR7, hTLR8 and hTLR9. *Eur. Cytokine Netw* 2000;11:372–378. [PubMed: 11022120]
6. Du X, Poltorak A, Wei Y, Beutler B. Three novel mammalian Toll-like receptors: gene structure, expression, and evolution. *Eur. Cytokine Netw* 2000;11:362–371. [PubMed: 11022119]
7. Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF. A family of human receptors structurally related to *Drosophila* Toll. *Proc. Natl Acad. Sci. USA* 1998;95:588–593. [PubMed: 9435236]
8. Takeda K, Takeuchi O, Akira S. Recognition of lipopeptides by Toll-like receptors. *J. Endotoxin Res* 2002;8:459–463. [PubMed: 12697090]
9. Takeuchi O, Kawai T, Sanjo H, et al. TLR6: a novel member of an expanding Toll-like receptor family. *Gene* 1999;231:59–65. [PubMed: 10231569]
10. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF- κ B by Toll-like receptor 3. *Nature* 2001;413:732–738. [PubMed: 11607032]

11. Kariko K, Ni H, Capodici J, Lamphier M, Weissman D. mRNA is an endogenous ligand for Toll-like receptor 3. *J. Biol. Chem* 2004;279:12542–12550. [PubMed: 14729660]
12. Hayashi F, Smith KD, Ozinsky A, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 2001;410:1099–1103. [PubMed: 11323673]
13. Johnson GB, Brunn GJ, Kodaira Y, Platt JL. Receptor-mediated monitoring of tissue well-being via detection of soluble heparan sulfate by Toll-like receptor 4. *J. Immunol* 2002;168:5233–5239. [PubMed: 11994480]
14. Lehnardt S, Schott E, Trimbuch T, et al. A vicious cycle involving release of heat shock protein 60 from injured cells and activation of Toll-like receptor 4 mediates neurodegeneration in the CNS. *J. Neurosci* 2008;28:2320–2331. [PubMed: 18322079]
15. Smiley ST, King JA, Hancock WW. Fibrinogen stimulates macrophage chemokine secretion through Toll-like receptor 4. *J. Immunol* 2001;167:2887–2894. [PubMed: 11509636]
16. Barrat FJ, Meeker T, Gregorio J, et al. Nucleic acids of mammalian origin can act as endogenous ligands for Toll-like receptors and may promote systemic lupus erythematosus. *J. Exp. Med* 2005;202:1131–1139. [PubMed: 16230478]
17. Diebold SS, Kaisho T, Hemmi H, Akira S, e Sousa C Reis. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 2004;303:1529–1531. [PubMed: 14976261]
18. Hemmi H, Kaisho T, Takeuchi O, et al. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nat. Immunol* 2002;3:196–200. [PubMed: 11812998]
19. Hemmi H, Takeuchi O, Kawai T, et al. A Toll-like receptor recognizes bacterial DNA. *Nature* 2000;408:740–745. [PubMed: 11130078]
20. Zhang D, Zhang G, Hayden MS, et al. A Toll-like receptor that prevents infection by uropathogenic bacteria. *Science* 2004;303:1522–1526. [PubMed: 15001781]
21. Yarovinsky F, Zhang D, Andersen JF, et al. TLR11 activation of dendritic cells by a protozoan profilin-like protein. *Science* 2005;308:1626–1629. [PubMed: 15860593]
22. Lundberg AM, Drexler SK, Monaco C, et al. Key differences in TLR3/poly I:C signaling and cytokine induction by human primary cells: a phenomenon absent from murine cell systems. *Blood* 2007;110:3245–3252. [PubMed: 17660379]
23. Matsumoto M, Funami K, Tanabe M, et al. Subcellular localization of Toll-like receptor 3 in human dendritic cells. *J. Immunol* 2003;171:3154–3162. [PubMed: 12960343]
24. Okun E, Griffioen KJ, Lathia JD, Tang SC, Mattson MP, Arumugam TV. Toll-like receptors in neurodegeneration. *Brain Res. Rev.* 2008DOI: 10.1016/j.brainresrev.2008.09.001(Epub ahead of print)Thorough overview of the expression of Toll-like receptors (TLRs) in the CNS.
25. Jack CS, Arbour N, Manusow J, et al. TLR signaling tailors innate immune responses in human microglia and astrocytes. *J. Immunol* 2005;175:4320–4330. [PubMed: 16177072]
26. Olson JK, Miller SD. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. *J. Immunol* 2004;173:3916–3924. [PubMed: 15356140]
27. Cameron JS, Alexopoulou L, Sloane JA, et al. Toll-like receptor 3 is a potent negative regulator of axonal growth in mammals. *J. Neurosci* 2007;27:13033–13041. [PubMed: 18032677]
28. Jackson AC, Rossiter JP, Lafon M. Expression of Toll-like receptor 3 in the human cerebellar cortex in rabies, herpes simplex encephalitis, and other neurological diseases. *J. Neurovirol* 2006;12:229–234. [PubMed: 16877304]
29. Lafon M, Megret F, Lafage M, Prehaud C. The innate immune facet of brain: human neurons express TLR-3 and sense viral dsRNA. *J. Mol. Neurosci* 2006;29:185–194. [PubMed: 17085778]
30. Ma Y, Haynes RL, Sidman RL, Vartanian T. TLR8: an innate immune receptor in brain, neurons and axons. *Cell Cycle* 2007;6:2859–2868. [PubMed: 18000403]
31. Mishra BB, Gundra UM, Teale JM. Expression and distribution of Toll-like receptors 11–13 in the brain during murine neurocysticercosis. *J. Neuroinflammation* 2008;5:53. [PubMed: 19077284]
32. Mishra BB, Mishra PK, Teale JM. Expression and distribution of Toll-like receptors in the brain during murine neurocysticercosis. *J. Neuroimmunol* 2006;181:46–56. [PubMed: 17011049]

33. Tang SC, Arumugam TV, Xu X, et al. Pivotal role for neuronal Toll-like receptors in ischemic brain injury and functional deficits. *Proc. Natl Acad. Sci. USA* 2007;104:13798–13803. [PubMed: 17693552]
34. Bsibsi M, Ravid R, Gveric D, van Noort JM. Broad expression of Toll-like receptors in the human central nervous system. *J. Neuropathol. Exp. Neurol* 2002;61:1013–1021. [PubMed: 12430718]
35. Bsibsi M, Persoon-Deen C, Verwer RW, Meeuwssen S, Ravid R, Van Noort JM. Toll-like receptor 3 on adult human astrocytes triggers production of neuroprotective mediators. *Glia* 2006;53:688–695. [PubMed: 16482523]
36. Farina C, Krumbholz M, Giese T, Hartmann G, Aloisi F, Meinl E. Preferential expression and function of Toll-like receptor 3 in human astrocytes. *J. Neuroimmunol* 2005;159:12–19. [PubMed: 15652398]
37. Morris GE, Parker LC, Ward JR, et al. Cooperative molecular and cellular networks regulate Toll-like receptor-dependent inflammatory responses. *FASEB J* 2006;20:2153–2155. [PubMed: 16935934]
38. O'Neill LA, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat. Rev. Immunol* 2007;7:353–364. [PubMed: 17457343]
39. Cao CX, Yang QW, Lv FL, Cui J, Fu HB, Wang JZ. Reduced cerebral ischemia-reperfusion injury in Toll-like receptor 4 deficient mice. *Biochem. Biophys. Res. Commun* 2007;353:509–514. [PubMed: 17188246]
40. Oyama J, Blais C Jr, Liu X, et al. Reduced myocardial ischemia-reperfusion injury in Toll-like receptor 4-deficient mice. *Circulation* 2004;109:784–789. [PubMed: 14970116]
41. Lu CY, Hartono J, Senitko M, Chen J. The inflammatory response to ischemic acute kidney injury: a result of the 'right stuff' in the 'wrong place'? *Curr. Opin. Nephrol. Hypertens* 2007;16:83–89. [PubMed: 17293682]
42. Zhai Y, Qiao B, Shen XD, et al. Evidence for the pivotal role of endogenous Toll-like receptor 4 ligands in liver ischemia and reperfusion injury. *Transplantation* 2008;85:1016–1022. [PubMed: 18408583]
43. Aprahamian CJ, Lorenz RG, Harmon CM, Dimmit RA. Toll-like receptor 2 is protective of ischemia-reperfusion-mediated small-bowel injury in a murine model. *Pediatr. Crit. Care Med* 2008;9:105–109. [PubMed: 17906593]
44. Arumugam TV, Okun E, Tang SC, Thundiyil J, Taylor SM, Woodruff TM. Toll-like receptors in ischemia-reperfusion injury. *Shock*. 2008DOI:10.1097/SHK.0b013e318193e333(Epub ahead of print)
45. Brown JM, Grosso MA, Terada LS, et al. Endotoxin pretreatment increases endogenous myocardial catalase activity and decreases ischemia-reperfusion injury of isolated rat hearts. *Proc. Natl Acad. Sci. USA* 1989;86:2516–2520. [PubMed: 2648406]
46. Franco PJ, Fernandez DC, Sande PH, et al. Effect of bacterial lipopolysaccharide on ischemic damage in the rat retina. *Invest. Ophthalmol. Vis. Sci* 2008;49:4604–4612. [PubMed: 18552395]
47. Tasaki K, Ruetzler CA, Ohtsuki T, Martin D, Nawashiro H, Hallenbeck JM. Lipopolysaccharide pretreatment induces resistance against subsequent focal cerebral ischemic damage in spontaneously hypertensive rats. *Brain Res* 1997;748:267–270. [PubMed: 9067475] First paper to demonstrate that pretreating with lipopolysaccharide results in ischemic tolerance.
48. Rosenzweig HL, Lessov NS, Henshall DC, Minami M, Simon RP, Stenzel-Poore MP. Endotoxin preconditioning prevents cellular inflammatory response during ischemic neuroprotection in mice. *Stroke* 2004;35:2576–2581. [PubMed: 15375302]
49. Rosenzweig HL, Minami M, Lessov NS, et al. Endotoxin preconditioning protects against the cytotoxic effects of TNF- α after stroke: a novel role for TNF- α in LPS-ischemic tolerance. *J. Cereb. Blood Flow Metab* 2007;27:1663–1674. [PubMed: 17327883] Explores the mechanism of TLR4-mediated preconditioning, focusing on TNF- α as a key player.
50. Bordet R, Deplanque D, Maboudou P, et al. Increase in endogenous brain superoxide dismutase as a potential mechanism of lipopolysaccharide-induced brain ischemic tolerance. *J. Cereb. Blood Flow Metab* 2000;20:1190–1196. [PubMed: 10950379]
51. Stevens SL, Ciesielski TM, Marsh BJ, et al. Toll-like receptor 9: a new target of ischemic preconditioning in the brain. *J. Cereb. Blood Flow Metab* 2008;28:1040–1047. [PubMed: 18183029] First publication to show that TLR9 is a target for preconditioning against ischemic damage.

52. Hua F, Ma J, Ha T, et al. Preconditioning with a TLR2 specific ligand increases resistance to cerebral ischemia/reperfusion injury. *J. Neuroimmunol* 2008;199:75–82. [PubMed: 18585792]Provides novel evidence that pretreatment with a ligand of another member of the TLR family, TLR2, can lead to ischemic tolerance.
53. Vogel SN, Fitzgerald KA, Fenton MJ. TLRs: differential adapter utilization by Toll-like receptors mediates TLR-specific patterns of gene expression. *Mol. Interv* 2003;3:466–477. [PubMed: 14993454]
54. Sen GC, Sarkar SN. Transcriptional signaling by double-stranded RNA: role of TLR3. *Cytokine Growth Factor Rev* 2005;16:1–14. [PubMed: 15733829]
55. Touil T, Fitzgerald D, Zhang GX, Rostami A, Gran B. Cutting Edge: TLR3 stimulation suppresses experimental autoimmune encephalomyelitis by inducing endogenous IFN- β . *J. Immunol* 2006;177:7505–7509. [PubMed: 17114417]
56. Marsh BJ, Stevens SL, Hunter B, Stenzel-Poore MP. Inflammation and the emerging role of the Toll-like receptor system in acute brain ischemia. *Stroke*. 2008DOI: 10.1161/STROKEAHA.108.534917 (Epub ahead of print)
57. Chakravarty S, Herkenham M. Toll-like receptor 4 on nonhematopoietic cells sustains CNS inflammation during endotoxemia, independent of systemic cytokines. *J. Neurosci* 2005;25:1788–1796. [PubMed: 15716415]
58. Stenzel-Poore MP, Stevens SL, King JS, Simon RP. Preconditioning reprograms the response to ischemic injury and primes the emergence of unique endogenous neuroprotective phenotypes: a speculative synthesis. *Stroke* 2007;38:680–685. [PubMed: 17261715]Posits a new theory, based on genomic data, on reprogramming the brain's response to cerebral ischemia.
59. Nawashiro H, Martin D, Hallenbeck JM. Neuroprotective effects of TNF binding protein in focal cerebral ischemia. *Brain Res* 1997;778:265–271. [PubMed: 9459543]
60. Jatana M, Giri S, Ansari MA, et al. Inhibition of NF- κ B activation by 5-lipoxygenase inhibitors protects brain against injury in a rat model of focal cerebral ischemia. *J. Neuroinflammation* 2006;3:12. [PubMed: 16689995]
61. Liu H, Xin L, Chan BP, Teoh R, Tang BL, Tan YH. Interferon- β administration confers a beneficial outcome in a rabbit model of thromboembolic cerebral ischemia. *Neurosci. Lett* 2002;327:146–148. [PubMed: 12098656]
62. Veldhuis WB, Derksen JW, Floris S, et al. Interferon- β blocks infiltration of inflammatory cells and reduces infarct volume after ischemic stroke in the rat. *J. Cereb. Blood Flow Metab* 2003;23:1029–1039. [PubMed: 12973019]
63. Veldhuis WB, Floris S, van der Meide PH, et al. Interferon- β prevents cytokine-induced neutrophil infiltration and attenuates blood—brain barrier disruption. *J. Cereb. Blood Flow Metab* 2003;23:1060–1069. [PubMed: 12973022]
64. Jin S, Kawanokuchi J, Mizuno T, et al. Interferon- β is neuroprotective against the toxicity induced by activated microglia. *Brain Res* 2007;1179:140–146. [PubMed: 17905201]

Figure 1. Preconditioning by redirecting Toll-like receptor signaling pathways

Using TLR4, which can signal through both the MyD88 and TRIF pathways, as an example, this figure depicts how the shift from a proinflammatory to an anti-inflammatory response may be achieved to provide neuroprotection. Signaling through the MyD88 pathway leads to the production of proinflammatory mediators, whereas signaling through the TRIF pathway both activates NF- κ B and induces Type I IFNs and IFN-inducible genes. Experiments using MyD88- and TRIF-deficient mice that have been preconditioned with lipopolysaccharide (LPS) have led to the hypothesis that TRIF is the key adaptor protein for the TLR4 response to LPS preconditioning, since MyD88-deficient mice can be preconditioned whereas TRIF-deficient mice cannot. In turn, signaling down the TRIF pathway leads to the activation of NF- κ B for proinflammatory responses and IRFs for anti-inflammatory responses. The initial TLR4

stimulation by LPS (solid line) may reprogram the subsequent TLR4 response to ischemia to one that activates IRFs and IFN production (dotted line). This reprogramming may be mediated by several pathway inhibitors (e.g., TRIM30 α), which are upregulated after LPS preconditioning as well as ischemic insult, and may act to destabilize the TAK1 complex, which in turn blocks NF- κ B activation.

IFN: Interferon; IRF: IFN-regulatory factors; TAB: Transforming growth factor-B binding protein; TIR: Toll—interleukin 1 receptor; TIRAP: TIR domain-containing adaptor protein; TLR: Toll-like receptor; TRAF: TNF-receptor-associated factor; TRIF: TIR domain-containing adaptor inducing IFN.