Review

Innate immune receptor Toll-like receptor 4 signalling in neuropsychiatric diseases

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A B S T R A C T

The innate immunity is a stereotyped first line of defense against pathogens and unspecified damage signals. One of main actors of innate immunity are the Toll-like receptors (TLRs), and one of the better characterized members of this family is TLR-4, that it is mainly activated by Gram-negative bacteria lipopolysaccharide. In brain, TLR-4 organizes innate immune responses against infections or cellular damage, but also possesses other physiological functions. In the last years, some evidences suggest a role of TLR-4 in stress and stress-related neuropsychiatric diseases. Peripheral and brain TLR-4 activation triggers sickness behavior, and its expression is a risk factor of depression. Some elements of the TLR-4 signaling pathway are up-regulated in peripheral samples and brain post-mortem tissue from depressed and suicidal patients. The “leaky gut” hypothesis of neuropsychiatric diseases is based on the existence of an increase of the intestinal permeability which results in bacterial translocation able to activate TLR-4. Enhanced peripheral TLR-4 expression/activity has been described in subjects diagnosed with schizophrenia, bipolar disorder and in autistic children. A role for TLR-4 in drugs abuse has been also proposed. The therapeutic potential of pharmacological/genetic modulation of TLRs signaling pathways in neuropsychiatry is promising, but a great preclinical/clinical scientific effort is still needed.

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1. Introduction

The innate immunity is the first line of host defence against pathogens, being phylogenetically conserved from Drosophila to mammals. It is considered a stereotyped, nonspecific response, as compared to adaptive immunity that is specific to each pathogen. One of main actors of innate immunity are the Toll-like receptors (TLRs), which belong to the super-family of Pattern Recognition Receptors (PRRs) that detect conserved pathogen-associated molecular patterns (PAMPs) (Takeda et al., 2003). PAMPs are not virulence factors but life-essential structures for the surveillance of the pathogen, and thus well conserved during evolution. The canonical example of PAMPs is the endotoxin lipopolysaccharide (LPS) from the outer membrane of Gram-negative bacteria (Poltorak et al., 1998a,b). LPS is recognized by the Toll-like receptor 4 (TLR-4), the first member of the family described in humans (Medzhitov et al., 1997).

TLRs also recognize multiple endogenous ligands, released as cellular or tissue danger/damage signals, named Damage-associated molecular patterns (DAMPs) or alarmins (Seong and Matzinger, 2004). In the case of TLR-4 the best described DAMPs are fibrinogen, heat shock proteins 60–70 (HSP60-70) and High-mobility-group box protein 1 (HMGB-1) (Piccinni and Midwood, 2010). The implication of DAMPs in the process called “sterile inflammation” supports the view of TLR-4 as a sentinel receptor that is activated also by some danger/damage signals in absence of pathogens (Mollen et al., 2006).

To date, 13 TLRs members have been described in mammals and 10 of them are functional in humans. The main human TLRs ligands and their biological functions relevant to neuropsychiatric disease are summarized in Table 1. In general, TLR-4 has multiple physiological functions, such as the trigger of fever and other acute phase responses in response to external noxia (Romanovsky et al., 2006), the proper resolution of the inflammatory process (Kigerl et al., 2007), the regulation of pain (Sauer et al., 2014) and the restoration of the central nervous system (CNS) homeostasis after injury (Griffiths et al., 2010).

2. TLR-4 signaling pathways

The intracellular elements of the TLR-4 signalling pathways may vary in function of the ligand involved. Also, a specific ligand may activate various pathways. In this section, the two main pathways activated by LPS, Myeloid differentiation primary response protein 88 (MyD88) dependent and independent, will be briefly described (Fig. 1).

Their common first step is the recognition of the lipid A-region of LPS by TLR-4 (Poltorak et al., 1998a,b). Circulating LPS is captured by the LPS binding protein (LBP) and the resultant dimer binds to a third protein named cluster of differentiation 14 (CD14), which is present in the membrane of innate immune cells or in plasma in its soluble form (Tobias et al., 1995). One function for CD14 is the transport of TLR-4 to lipid rafts regions of the cell membrane, amplifying its signalling (Triantafilou et al., 2002).

The next step is the recruitment of the co-receptor myeloid differentiation factor-2 (MD-2) (Shimazu et al., 1999). The binding with MD-2 is fundamental for facilitating the translocation of TLR-4 to the cellular membrane (Nagai et al., 2002). Once stimulated, the complex TLR-4/MD-2 is endocytosed (Fujihara et al., 2003). Once the heterotrimer CD14/TLR-4/MD-2 is associated to LPS, two alternative signalling pathways can be triggered (da Silva et al., 2001): MyD88-dependent and MyD88-independent pathways.

2.1. MyD88-dependent pathway

TLR-4 binds to the adapter protein MyD88 through its cytoplasmic domain TIR (toll-interleukin 1 (IL-1) receptor) and then recruits the IL-1 receptor-associated kinase IRAK4. IRAK4 joins and activates the NF-kappa-B (NF-kB) protein through the TIR domain, constituting the MyD88-dependent pathway (Medzhitov and Janeway, 2002). IRAK4 joins and activates the NF-kappa-B (NF-kB) protein through the TIR domain, constituting the MyD88-dependent pathway (Medzhitov and Janeway, 2002). IRAK4 joins and activates the NF-kappa-B (NF-kB) protein through the TIR domain, constituting the MyD88-dependent pathway (Medzhitov and Janeway, 2002).

2.2. MyD88-independent pathways

These alternative pathways are dependent on the activation of some adaptor proteins, such as TIR-domain-containing adapter-inducing interferon-β (TRIF) and the translocating chain-associated membrane (TRAM) protein (Hyun et al., 2013). These two proteins dimerize in a heterodimer TRAM–TRIF that is capable to activate the TRIF–TRAM–TIR domain pathway, already commented. Alternatively, TRIF could form the homodimer TRIF–TRIF and trough the activation of TBK1 regulates the expression of Interferon regulatory factor 3 (IRF3) and interferon 1β (IFN-1β). IFN-1β binds to its membrane receptors activating the transcription factor Signal transducer and activator of transcription (STAT).

Multiple cellular mechanisms exist to regulate TLR-4 over-activation in pathological conditions. The main one is the
production of endogenous inhibitors, such as the ubiquitin ligase TRIAD3A that promotes the ubiquitination and posterior degradation of TLR-4 (Chuang and Ulevitch, 2004), or the homologous TLR-4 protein RP105, that blocks the TLR-4 signalling at cellular membrane level (Divanovic et al., 2005). Recent studies are focusing in other relevant mechanisms of regulation, such as the post-translational modification of TLR-4 signalling through the acetylation of lysine residues (Hu et al., 2013).

### 3. TLR-4 expression in the central nervous system (CNS)

Although CNS has long been considered an immune-privileged organ, this immune status is far from absolute. CNS is able to organise innate immune responses against infections or cellular damage through the activation of TLRs (Bisbi et al., 2002). In fact, TLR-4 is highly expressed in microglia, the resident immune cells in the CNS (Lehnardt et al., 2003). Also, neurons (Tang et al., 2008), astroglia (Jou et al., 2006), oligodendroglia (Kigerl et al., 2007) and cerebral vascular endothelium also express TLR-4, although in a lesser extent (Nagyosi et al., 2010).

Regarding the brain areas in which TLR-4 expression is more representative it is worth to mention from a functional point of view the high expression found in brain areas lacking of blood brain barrier (BBB), circumventricular organs, and in the plexus choroidales and leptomeninges (Lafamme and Rivest, 2001; Lacroix et al., 1998). There, TLR-4 could be monitoring and orchestrating peripheral immune responses at CNS level. In addition, a cross-talk between brain parenchyma cells and endothelium exists at the neurovascular level: neuronal and astrocytic TLR-4 signalling induces brain endothelial activation and neutrophil transmigration in mouse mixed glial, neuronal or endothelial cell cultures incubated in presence of LPS (Leow-Dyke et al., 2012). Also, TLR-4 regulates MAPK and Jak1/Stat1 signalling pathways in purified rodent brain astrocyte cultures after LPS (Gorina et al., 2011). Thus, neurons and astrocytes detect peripheral infection or DAMPs and are able to initiate CNS inflammation through a TLR-4-dependent mechanism.

In summary, the activation of TLR-4 at CNS level occurs by several mechanisms: (1) circulating leukocytes expressing TLR-4 release inflammatory molecules capable of activating specific brain areas; (2) direct activation of the TLR-4 present in the brain circumventricular organs and other leaky structures, such as the choroid plexus and leptomeninges; (3) direct activation of TLR-4 expressed by endothelial and perivascular cells forming the blood–brain barrier; and (4) activation of the TLR-4 expressed by microglia, astroglia or neurons surrounding brain microvasculature. All of these are non-excluding mechanisms.

### 4. Patho-physiological roles of TLR-4 in the CNS

#### 4.1. TLR-4 signalling and neurotransmission

Activation of TLR-4 attenuates GABA synthesis and postsynaptic GABA receptor activities in astrocytes of rodent spinal slices in an IL-1β release-dependent mechanism (Yan et al., 2015). TLR-4 expression is regulated by GABA(A) α2 receptor in the central amygdala in a model of binge alcohol drinking, and its activation plays an important role in the acute ethanol-induced potentiation of GABAergic transmission in this brain area (Liu et al., 2011; Bajo et al., 2014).

On the other hand, TLR-4 activation has been implicated in glutamate-induced excitotoxicity after NMDA receptor over-activation after stroke (Qiu et al., 2008) or LPS (Glezer et al., 2003) in vivo. In a possible TLR-4 upstream related mechanism, the damaging signal HMGBl potentiates NMDA receptor-dependent cell responses binding to TLR4 in isolated hippocampal nerve terminals and in a neuroblastoma cell line in vitro (Pedrazzi et al., 2012).
Regarding biogenic amines, it has been recently described that TLR-4 regulates the mesolimbic dopamine system that amplifies opioid-induced elevations in extracellular dopamine levels in rats submitted to a model of opioid self-administration (Hutchinson et al., 2012). In addition, TLR-4 activation with cocaine produces an increase in the extracellular content of dopamine in the nucleus accumbens, as well as cocaine reward and reinforcement in rodents (Northcutt et al., 2015). Recently it has been suggested that central TLR-4 could be a negative regulator of the sympathetic drive in hypertension-induced models (Dange et al., 2015, 2014).

Some authors have hypothesized that the inflammatory consequences of glial TLR-4 activation could influence serotonergic neurotransmission, inhibiting serotonin synthesis through the induction of the enzyme indoleamine-2,3-dioxygenase (IDO). IDO catalyzes the degradation of L-tryptophan producing quinolinic acid and 3-hydroxy-kynurenine, which can further result in excitotoxicity (O’Connor et al., 2009). This hypothesis could be relevant in the pathophysiology of depression, where altered IDO activity and kynurenine metabolites brain content have been described (Maes et al., 2011; Myint et al., 2007; Savitz et al., 2015).

Thus, TLR-4 signalling has been implicated in the regulation of the neurotransmission pathways related to the pathophysiology of major psychiatric and neurological diseases.

4.2. TLR-4 and neuroinflammation in CNS pathological conditions

As the inflammation/innate immune system activation is common to several CNS pathologies, TLR-4 has been implicated in...
their pathophysiology and its pharmacological/genetic manipulation has been suggested as a putative therapeutic target (see Table 3). Specifically, TLR-4 plays a decisive role in the initiation of the neuroinflammatory process dependent of NF-κB activation in microglia, leading to the transcription of multiple pro-inflammatory genes (MAP kinases, cytokines, chemokines, proinflammatory enzymes) in pathologies such as stroke, traumatic brain injury, neurodegeneration induced by alcohol abuse, Alzheimer’s disease (AD), Parkinson’s disease (PD), Multiple Sclerosis (MS) and chronic pain pathologies (Sauer et al., 2014; Buchanan et al., 2010; Trotta et al., 2014).

4.3. Other TLR-4 specific effects in CNS pathological conditions

Although neuroinflammation is common to all these pathologies, each one has particular characteristics and pathophysiological mechanisms that could be affected or regulated by TLR-4. This is the case for stroke, in which TLR-4 has been recently implicated in the regulation of blood-spinal cord barrier permeability (Li et al., 2014), cell migration and cortical neurogenesis after focal cerebral ischemia (Moraga et al., 2014). Similarly, an association between TLR-4 and neural stem cell proliferation in hippocampus has been reported following traumatic brain injury (TBI) in mice (Ye et al., 2014). Also, in TBI, TLR-4 activation contributes to the formation of cerebral oedema in a HMB1-related mechanism (Laird et al., 2014).

Regarding Alzheimer’s disease, TLR-4 could show a predominant neuroprotective role regulating the uptake and phagocytic removal of Aβ plaques by microglia (Tahara et al., 2008), although a TLR-4-driven neurotoxicity has been also described (Trotta et al., 2014). A similar neuroprotective role has been suggested for TLR-4 in Parkinson’s disease, regulating the clearance of alpha-synuclein in a transgenic mouse model featuring oligodendroglial alpha-synuclein inclusions and loss of midbrain dopaminergic neurons (Stefanova et al., 2007). The TLR-4 dependent phagocytic capacity of microglia could be extended to degenerative axons in multiple sclerosis (MS) and amyotrophic lateral sclerosis, creating a permissive environment for axonal outgrowth (Rajbhandari et al., 2014). In addition, at least in MS mouse experimental models, TLR-4/MyD88 is required in characteristic pathological hallmarks of MS, such as the activation of myeloid dendritic cells and differentiation of TH17 lymphocytes (rev. in Marta, 2009).

Finally, TLR-4 has been also implicated in the regulation of altered behaviours including learning, memory and anxiety changes in animal models of CNS pathologies (rev. in Okun et al., 2011). The influence of the innate immune system response to infection on behaviour is, by far, the most studied aspect in this field. Thus, LPS-derived activation of TLR-4 produces sickness and depressive-like behaviour through pro-inflammatory cytokines, such as IL-1β, TNF-α, IL-6 and IFN-γ (rev. in McCusker and Kelley, 2012). Several mechanisms have been proposed to explain how the brain monitors peripheral immune signals to mount the proper behavioural responses (rev. in McCusker and Kelley, 2008): one pathway involves locally produced cytokines activating primary afferent nerves; a second pathway is a humoral pathway in which TLRs expressed on endothelium and macrophage-like cells residing in the circumventricular organs and the choroid plexus respond to circulating pathogen-associated molecular patterns by producing proinflammatory cytokines; a third pathway comprises proinflammatory cytokine transporters at the blood brain barrier; and finally, a fourth pathway involves cytokine receptors located on perivascular macrophages and endothelial cells of brain vasculature that transduce the signals into brain parenchyma through a prostaglandin (PGE2)-dependent mechanism.

Thus, in addition to its pro-inflammatory role, TLR-4 signalling pathway regulates several specific processes taking place in CNS pathological conditions, such as cellular plasticity, microglial phagocytic activity, activation of the acquired immune system and behaviour that could be considered putative therapeutic targets in the future.

5. TLR-4 and stress-related neuropsychiatric diseases

According to the diathesis-stress model, stress is essential to the development of several neuropsychopathologies, unmasking the underlying individual predisposition to the disorders (Monroe and Simons, 1991). In the field of innate immunity, several of the first evidences supporting the involvement of TLRs in neuroinflammation were obtained in stress based experimental models of neuropsychiatric diseases (Table 2).

5.1. TLR-4 and stress

After the demonstration of TLR-4 expression in the human adrenal cortex (Bornstein et al., 2004), it was confirmed that increased systemic LPS induced the adrenal synthesis of cortisol in a TLR-4-dependent mechanism through the activation of the NF-κB/COX-2/PGE2 pathway (Vakharia and Hinson, 2005; Martínez et al., 2011). Corroborating these findings, functional TLR-4 KO mice presented structural and functional alterations in the adrenal glands (hypertrophy, increased of basal levels of corticosterone in plasma and unresponsiveness to LPS stimulation) (Zacharowskiet al., 2006). At CNS level, Gosselin and Rivest (2008) suggested the possibility for circulating LPS to produce an HPA axis-driven release of glucocorticoids through the direct activation of the TLR-4 expressed in the circumventricular organs, being this intracellular mechanism dependent of MyD88 activation.

Recently it has been shown that different protocols of stress exposure (repeated social defeat, restraint stress and chronic mild stress) up-regulated TLR-4 mRNA and protein levels in the rat brain prefrontal cortex (PFC) (Gárate et al., 2011, 2013, 2014). In particular, the stress-induced NF-κB activation, iNOS and COX-2 upregulation, and cellular oxidative/nitrosative damage are reduced when the TLR-4 pathway is defective (by pharmacological/genetic manipulation).

A direct role for LPS has been identified as possible mechanism/s implicated in stress-induced TLR-4 activation. Approaches using antibiotic intestinal decontamination suggested a role of bacterial translocation (LPS and LB increased plasma levels, as well as presence of colony forming units of living indigenous microflora in mesenteric lymph nodes, liver and spleen) on TLR-4 signalling pathway activation in the PFC after stress exposure as a result of stress-induced intestinal barrier dysfunction (Gárate et al., 2013, 2014). This proposed mechanism, known as “leaky gut”, also takes place in depressed and chronic fatigue syndrome patients, and has been related to their pathophysiology (Maes et al., 2008).

Although this bacterial translocation is responsible, at least in part, for the stress induced TLR-4 up-regulation in the brain, several other non-excluding mechanisms should not be ruled out. This is the case for some DAMPs, such as HSP60-70 or HGBP-1 (Fleschner, 2013), which increase in rat brain PFC after stress exposure (MacDowell et al., 2015). During situations of stress-induced cellular damage these molecules are released to the extracellular space and alert the immune system in order to promote repair and direct trafficking toward the damaged tissue, triggering the inflammatory response driven by TLR-4 (Liu et al., 2014).

The excitatory amino acid glutamate, which is rapidly released after stress exposure in the PFC (Moghaddam, 1993) has been also directly related to the regulation of TLR-4 via a N-methyl-D-aspartate receptor-dependent mechanism after increasing systemic LPS (Glezer et al., 2003). Other stress mediators, such
as epinephrine/norepinephrine/β2 adrenergic receptor and corticotrophin releasing factor also regulate TLR-4 expression in colon and immune cells (Chaniotou et al., 2010; Kizaki et al., 2008).

In summary, stress exposure elicits a NF-κB proinflammatory response in brain driven by a prior activation of TLR-4, as occurs in the pathophysiology of several neurological/neurodegenerative diseases (as previously commented) (revs. in Buchanan et al., 2010; Trotta et al., 2014).

All of these results suggest that TLR-4 represents an important regulatory factor in the physiological response to stress and also support the possibility for pharmacological manipulations of this pathway in order to minimize brain oxidative and inflammatory damage after stress exposure and in stress-related psycho/neuropathologies. However, further investigations are needed to address whether the loss or inhibition of TLR-4 is beneficial or predominantly harmful in different pathological scenarios (rev. in Ishii et al., 2006).

5.2. TLR-4 and depression

The first evidences suggesting a role for TLR-4 in depression came from animal models mimicking a bacterial infection through the systemic administration of endotoxins. In this way, peripheral TLR-4 activation with LPS causes changes in motivational state and can trigger sickness behaviour in animal models (Hines et al., 2013), characterized by increased anhedonia, lethargy, loss of locomotion and anorexia (rev. in Dantzer et al., 2008). This depressive behaviour is not exclusive of LPS, in fact, TLR-4 up-regulation in the brain has been directly related to depression- and anxiety-like behaviours induced by hypercholesterolemia in mice (Strekalova et al., 2015).

Increasing evidence from the clinical arena suggests that TLRs are involved in the pathophysiology of major depressive disorder (MDD). Recently, TLR-4 mRNA expression in peripheral blood has been identified as an independent risk factor related to severity of MDD (17-item Hamilton Depression Rating Scale) in a cohort of 30 patients and 29 matched controls (Hung et al., 2014). In addition, some elements of the TLR-4 signalling pathway, such as TRIF and MyD88, were up-regulated at mRNA level in peripheral blood mononuclear cells (PBMCs) of 38 depressed subjects (Hajebrahimi et al., 2014). These peripheral alterations of TLR-4 signalling pathway have been corroborated in human brain post-mortem tissue (dorsolateral prefrontal cortex) from depressed and suicidal patients (Pandey et al., 2014).

Other authors have also described alterations on the TLR-4 and proinflammatory NF-κB mRNAs levels in the PBMCs of 50 patients with first-episode MDD and have explored the “leaky gut” hypothesis of depression. In particular, an increase in 16S rRNA subunit of intestinal microbiota in plasma of the participants has been identified. More importantly, bacterial translocation/TLR-4/NF-κB activation was decreased after cognitive psychotherapy (Keri et al., 2014).

As already commented in the previous section, some experimental models of stress induce intestinal dysfunction followed by an increase of the intestinal permeability which results in bacterial translocation (Leonard and Maes, 2012). Thus, stress exposure could cause the presence of circulating LPS which can activate brain TLR-4 through multiple pathways, inducing a neuroinflammatory response. Remarkably, the “leaky gut” also takes place in patients with chronic depression, suggesting that depression is accompanied by bacterial translocation which would be related

<table>
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Table 3
Pharmacological modulation of TLR4 signalling in experimental models of CNS pathologies.

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<tr>
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</tr>
<tr>
<td>Isoflurane preconditioning</td>
<td>Inhibition of the upregulation of TLR4 downstream molecules</td>
<td>Neuroprotection</td>
<td>Oxidative stress</td>
<td>Sun et al. (2013)</td>
</tr>
<tr>
<td>Budesonide</td>
<td>Inhibition of TLR-4 up-regulation</td>
<td>Antiinflammatory</td>
<td>Chronic asthma-induced neuroinflammation</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Polyinosinic-polycytidylic acid (poly(I:C))</td>
<td>Downregulation of TLR4 signaling via TLR3.</td>
<td>Neuroprotection</td>
<td>Cerebral ischemia/reperfusion injury</td>
<td>Wang et al. (2014b)</td>
</tr>
<tr>
<td>Ligustilide</td>
<td>Inhibition of TLR4/peroxiredoxin 6 signaling</td>
<td>Neuroprotection</td>
<td>Brain injury in focal cerebral ischemia/reperfusion</td>
<td>Kuan et al. (2014)</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Inhibition of the TLR4/MyD88/NF-κB signaling pathway</td>
<td>Antiinflammatory</td>
<td>Traumatic brain injury</td>
<td>Zhu et al. (2014)</td>
</tr>
<tr>
<td>Pseudoginsenoside-F11</td>
<td>Inhibition of TLR4-mediated TAK1/IKK/NF-κB, MAPKs and Akt signaling pathways</td>
<td>Neuroprotection</td>
<td>LPS-activated microglia</td>
<td>Wang et al. (2014c)</td>
</tr>
<tr>
<td>Candesartan</td>
<td>Downregulation of the TLR signalling cascade</td>
<td>Neuroprotection</td>
<td>Ischemic brain damage</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Glycyrrhizin</td>
<td>Downregulation of the TLR signalling cascade</td>
<td>Neuroprotection</td>
<td>Oxidative stress</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>Downregulation of the TLR4/NF-κB signaling pathway</td>
<td>Neuroprotection</td>
<td>Oxidative stress</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>Inhibition of TLR4 signal transduction</td>
<td>Neuroprotection</td>
<td>Oxidative stress</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Shikonin</td>
<td>Attenuation of TLR4 expression</td>
<td>Antiinflammatory</td>
<td>Oxidative stress</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Lipopolysaccharide produced by Rhodobacter sphaeroides (LPS-RA)</td>
<td>TLR-4 antagonism</td>
<td>Neuroprotection</td>
<td>Oxidative stress</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Isoquercetin</td>
<td>Suppression of TLR4-NF-κB signal pathway</td>
<td>Neuroprotection</td>
<td>Oxidative stress</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Melatonin</td>
<td>Suppression of TLR4-signal pathway</td>
<td>Neuroprotection</td>
<td>Experimental subarachnoid hemorrhage</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Melatonin</td>
<td>Suppression of TLR4-signal pathway</td>
<td>Neuroprotection</td>
<td>Ischemic stroke</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Propofol</td>
<td>Downregulation of TLR4, MyD88, and NF-κB</td>
<td>Neuroprotection</td>
<td>Oxidative stress</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Albumin</td>
<td>Downregulation of TLR4</td>
<td>Neuroprotection</td>
<td>Experimental subarachnoid hemorrhage</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Oxytetracin</td>
<td>Downregulation of TLR4 and NF-κB</td>
<td>Neuroprotection</td>
<td>Oxidative stress</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Schisandrin B</td>
<td>Inhibition of TLR4, MyD88, and NF-κB</td>
<td>Neuroprotection</td>
<td>Oxidative stress</td>
<td>Zhao et al. (2014c)</td>
</tr>
<tr>
<td>Pituitary adenylate cyclase-activating polypeptide +)-Naloxone</td>
<td>TLR4 signalling inhibitor</td>
<td>Protection against neuropathic pain</td>
<td>Chronic neuropathic pain</td>
<td>Lewis et al. (2012)</td>
</tr>
<tr>
<td>OsPAPC</td>
<td>TLR-4 and 2 antagonist</td>
<td>Antiinflammatory</td>
<td>Stress (inevitable tailshock)</td>
<td>Weber et al. (2013),</td>
</tr>
<tr>
<td>Ginkgolide B</td>
<td>Down regulation of TLR4 and NF-κB</td>
<td>Antiapoptotic</td>
<td>Traumatic brain injury</td>
<td>Yu et al. (2012)</td>
</tr>
<tr>
<td>Luteolin</td>
<td>Down regulation of TLR4 and NF-κB</td>
<td>Neuroprotection</td>
<td>Middle Cerebral Artery Occlusion</td>
<td>Qiao et al. (2012)</td>
</tr>
<tr>
<td>Wogonin</td>
<td>Inhibition of TLR4/NF-κB signaling path</td>
<td>Neuroprotection</td>
<td>Middle Cerebral Artery Occlusion</td>
<td>Qiao et al. (2012)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>Attenuation of TLR4/NF-κB signaling path</td>
<td>Neuroprotection</td>
<td>Subarachnoid hemorrhage</td>
<td>Wang et al. (2011)</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>Attenuation of TLR4/NF-κB signaling path</td>
<td>Neuroprotection</td>
<td>Contusion</td>
<td>Chen et al. (2009)</td>
</tr>
</tbody>
</table>

Compounds eliciting TLR-4 non-specific effects in different experimental conditions are in bold.

to the inflammatory pathophysiology of the disease (Leonard and Maes, 2012; Maes et al., 2008, 2012).

Based on all these findings, the use of antibiotics as coadjuvant drugs for the treatment of MDD deserves further consideration, always taking into account the detrimental effects of chronic antibiotic use on mucosal integrity and the putative changes produced in the equilibrium of commensal microbiota (Levy, 2000). Alternatively, the use of other evidence-based treatments targeting the “leaky gut”, such as glutamine, has been suggested (Maes and Leunis, 2008).

5.3. TLR4 and schizophrenia

Findings suggesting an imbalance in TLR4 signalling have been also found in schizophrenia. Enhanced peripheral TLRs responses have been demonstrated in 40 patients with schizophrenia, producing a massive release of IL-6 or TNF-α in stimulated whole

...
blood with TLR2 and 4 agonists (McKernan et al., 2011). Other authors found increased TLR-4 expression in the monocytes of 31 schizophrenia patients compared to matched controls (Muller et al., 2012). Interestingly, these monocytes are less reactive to viral infection, supporting a role for TLR-4 and other sentinel receptors of the TLR family in the low-grade peripheral immune dysfunction and inflammatory imbalance found in psychosis (Garcia-Bueno et al., 2014; Leza et al., 2015).

One of the current areas of interest in psychiatry is the neurodevelopmental theory of psychosis. Multiple studies have established that TLRs are expressed in the developing brain and play a significant role in neurodevelopmental and plasticity processes (Okun et al., 2011). Activation of some TLRs subtypes inhibits cortical neurogenesis, neurite outgrowth and behavioural abnormalities in the offspring (Venkatasubramanian and Deb Nath, 2013). In addition, the absence of TLR-4 resulted in enhanced in vivo proliferation and differentiation of neural stem/progenitor cells in hippocampus (Rolls et al., 2007) and the systemic stimulation of TLR-2 produced a reduction on grey and white matter volume and hippocampal neuron density as well as increasing the number of microglial cells on neonatal mouse brain (Du et al., 2011).

Multiple lines of evidence have significantly been attributed to inflammatory and oxidative/nitrosative pathways in brain in schizophrenia (Meyer et al., 2011). A considerable effort is currently taking place in order to link TLRs with prenatal infections, inflammation, obstetric complications, oxidative/nitrosative stress, neurodegenerative and cognitive changes within the pathogenesis of schizophrenia (Venkatasubramanian and Deb Nath, 2013). There is growing evidence from animal models supporting a decisive role for pre- and perinatal infections in the induction of maternal immune activation and oxidative/nitrosative stress that can lead to neurodevelopmental damage and behavioural abnormalities in the progeny in rodents (Venkatasubramanian and Deb Nath, 2013). TLR-4 could participate decisively in these priming alterations of the immune system. In fact, it has been described that the activation of TLRs by infection affects the feto-maternal immune response and causes behavioural abnormalities of the descendants (De et al., 2010; Abrahams, et al., 2004).

The putative activation of TLR-4 in schizophrenia could be related to various alternative mechanisms, i.e. patients with schizophrenia quite often present gut problems and some risk factors for schizophrenia are related to the gastrointestinal system, such as gluten and milk casein hypersensitivity or Toxoplasma gondii infection (Dickerson et al., 2010; Severance et al., 2014). In addition, recent studies indicate an altered microbiota in schizophrenia and signs of intestinal inflammation, increased intestinal barrier permeability and bacterial translocation (Severance et al., 2013). As in MDD, such processes might trigger innate immunity through TLR-4 stimulation.

Pharmacological approaches have been also implicated brain TLR-4: it has been recently demonstrated a regulatory role of the antipsychotic paliperidone in the activation of TLR-4 of rats submitted to acute/chronic restraint stress. Paliperidone pre-treatment regulated stress-induced increased intestinal inflammation/dysfunction, plasma LPS levels and DAMPs activation (MacDowell et al., 2015).

In summary, the evidence for a role of TLR-4 in psychosis is still limited although there are attractive emerging hypotheses to explore. Preclinical studies with novel animal models of psychosis, clinical longitudinal and genetic studies with a larger number of subjects and the use of brain post-mortem tissue samples are needed to corroborate the findings reported so far in humans at peripheral level.

5.4. TLR-4 and other psychiatric diseases

Enhanced peripheral TLR-4 responses (in terms of increased proinflammatory cytokine release in TLRs agonists-stimulated whole blood samples) have been also demonstrated in subjects with bipolar disorder (BD) (McKernan et al., 2011). Recently, a genetic association between BD and TLR-4 has been described, finding that TLR-4 SNPs polymorphisms rs1927914 AA and rs11536891 TT are more frequent in BD patients than in controls (Oliveira et al., 2014). It has been also described an increased monocyte response (IL-1β release) to LPS in children with autism spectrum disorders (Enstrom et al., 2010), and increased TLR-4 gene expression in the immune cells of patients with chronic fatigue syndrome (Nijis et al., 2014). This way, the evidence of the role of TLR-4 in other psychiatric diseases is growing.

5.5. TLR-4 and drug abuse

5.5.1. TLR-4 and alcohol

The relationship between ethanol exposure and TLR-4 activation in macrophages was first described in alcohol-induced liver injury and disease in murine animal models and humans (Mandrekar et al., 2002; Uesugi et al., 2001; Oak et al., 2006). Once TLR-4 expression was firmly demonstrated in brain cell populations, the effects of ethanol at CNS level were also studied. First, the inflammatory effects of ethanol trough TLR-4 activation were demonstrated in vitro and in vivo studies using astrocytes (Blanco et al., 2005; Valles et al., 2004) and microglia (Fernandez-Lizarbe et al., 2008). Later on, ethanol–TLR-4 effects on myelin disruption, white matter and neurogenesis loss, and behavioural and cognitive dysfunctions associated with alcohol-induced neuroinflammatory damage were demonstrated (Pascual et al., 2011; Vetreno et al., 2015; Wu et al., 2012; Vetreno and Crews, 2012). An interesting role for TLR-4 in ethanol-induced alterations in protein degradation pathways (ubiquitin–proteasome and autophagy–lysosome) has also been recently described (Pla et al., 2014). Some of these effects have been also shown in human brain post-mortem tissue strengthen the existing evidence (Crews et al., 2013).

As it has been commented for depression, the mechanisms underlying ethanol activation of TLR4 receptors may include, apart from the direct activation, an increase in the intestinal permeability to endotoxins. Elevated levels of blood LPS and “leaky gut” have been reported in alcohol-dependent animals and alcohol-dependent non-cirrhotic humans (Adachi et al., 1991; Leclercq et al., 2012).

5.5.2. TLR-4 and opioids

The first description that opioids (morphine) could activate TLR-4 was made using in vitro and in vivo models, and by means of in silico techniques (Hutchinson et al., 2010). Then, it has been shown that opioids could activate TLR-4 in an specific binding site, inducing a strong proinflammatory gial activation and producing unwanted deleterious effects such as opioid tolerance, dependence, reward, respiratory depression and pain (Hutchinson et al., 2012; Watkins et al., 2009; Hutchinson et al., 2009). More recently, it has been described that the activation of CNS endothelial cells by opioid-induced TLR4 signalering also induces proinflammatory-driven effects (increased pro-inflammatory cytokine and prostaglandins release) at multiple levels, even affecting the normal pain response (Grace et al., 2014).

5.5.3. TLR-4 and cocaine

Recently, it has been discovered that cocaine reward and reinforcement requires the activation of Toll-like receptor 4 in
microglia in a cocaine self-administration model in rats (Northcutt et al., 2015).

In conclusion, there are accumulating findings supporting a role for TLR-4 in the detrimental effects produced by the abuse of particular substances on CNS structure and function (especially relevant in the case of ethanol and opioids), but also in the mechanisms governing addictive behaviour.

6. Genetic/pharmacological manipulation of TLR-4

Several genetically manipulated animal models have been developed. The great majority is based on a spontaneous mutation occurring in mice at the LPS response locus (later identified as a mutation in the TLR-4 gene, TLR-4Lps-d) (Poltorak et al., 1998a,b). These TLR-4 deficient mice (C3H/HeJ) exhibit defects in responses to LPS, including pro-inflammatory cytokine production, susceptibility to bacterial infections, ischemia-induced tissue injury, myocardial infarction, neurodegeneration and cancer related immunities (Carty and Bowie, 2011). Other genetic model is the one comprising the TLR-4 null mutant mice generated at the Osaka University, (Hoshino et al., 1999).

There are a relatively reduced number of studies exploring the effects of TLR-4 pharmacological inhibition in CNS pathologies at preclinical level. As previously mentioned, before establishing a proper pharmacological strategy based on the modulation of TLR-4 pathway, there should be an increase in the knowledge of the physiological roles of TLR-4 signalling pathways. As occurs with other CNS focused drugs, a very relevant issue to consider is the CNS bioavailability and specificity regarding the ability of possible studied agents to cross the blood–brain barrier. TLR-4 is widely expressed in the organism, and it is difficult to know whether the possible effects of drugs are at CNS level, periphery, or both. Further studies with specific TLR-4 knock-out mice for each compartment/cellular type expressing this receptor are needed to resolve this issue. Finally the specificity of the compound for one or other member of TLR family should be also studied.

Obviously, the strategies used for the inhibition of TLR-4 pathway could be the use of molecules that directly bind to TLR-4 or to the TLR-4–MD-2 complex, or by interacting with the other proteins involved in LPS sensing and TLR-4 activation, namely LBP, CD-14, MD-2 and MyD88 (Peri and Piazza, 2012).

The most used TLR-4 antagonist is TAK-242 (ethyl (6R)-6-[N-(2-chloro-4-fluorophenyl) sulfonyl]cyclohex-1-ene-1-carboxylate), a specific inhibitor of TLR-4 that blocks the intracellular domain TLR Toll/IL-1 receptor without affecting the extracellular docking with LPS (Matsumaga et al., 2011). At CNS level, TAK-242 administration reduced the inflammatory damage produced following ischemia/reperfusion injury (Li et al., 2014; Hua et al., 2015), experimental brain injury (Wang et al., 2013b; Zhang et al., 2014a) and acute restraint stress-induced neuroinflammation (Gárate et al., 2014) in rats.

There are other compounds capable to block TLR-4 signalling pathway at multiple levels that have been used in vitro and in vivo neuropathological models (see Table 3). The selected compounds elicit “neuroprotective” effects that could be relevant for the treatment of the main psychiatric diseases. However, it is important to remark that most of these compounds are not specific for TLR-4, and they are also able to regulate otherTLRs.

The use of siRNAs targeting TLR-4 is a novel and interesting approach that it is effective in chronic neuropathic pain models (Wu et al., 2010), neonatal brain hypoxia (Yao et al., 2013) and alcohol-induced neuroinflammation and brain damage (Alfonso-Loeches et al., 2010). Recently, it has been described that endogenous MicroRNA-181c negatively regulates the inflammatory response in oxygen-glucose-deprived microglia by targeting Toll-like receptor 4 (Zhang et al., 2015).

In a similar approach, the injection of Tat-TLR-4 interfering peptides (Lojarro et al., 2005) prevented LPS-induced microglia activation, cytokine production and sickness behaviour (Hines et al., 2013) and the administration of the TLR-4 blocker viral inhibitory peptide (VIPER) reduces the inflammatory response within the paraventricular nucleus of hypothalamus in a genetic model of hypertension (Dange et al., 2015).

7. Future investigations and conclusion

These are some areas of study that deserve attention in the near future:

(a) To go in depth in the knowledge of the elements of the TLR-4 signalling pathways for the future design and use of molecules capable to block TLR-4 activation at multiple and/or more specific levels (MD-2 structure, MyD88 vs TRIF/TRAM-mediated pathways).

(b) Following the paradigm considering that TLR-4 is not only a PAMPs receptor but also a sentinel of cellular damage, the study of putative and already described DAMPs is an open field to investigate new therapeutic targets. Possible excellent candidates to survey are HSP70 and HGBM1. HSP70 is a chaperone involved in neurodevelopment and neuroprotection and its defective production caused by stress during neuronal development could have a role in the pathophysiology of psychotic disease (Bates et al., 1996). HGBM-1 is a chromatin-binding protein, which facilitates the transcription on genes involved in neurite outgrowth and cell migration (Thomas and Travers, 2001) and also serves as a risk factor for memory impairment, chronic neurodegeneration, and progression of neuroinflammation (Fang et al., 2012).

(c) There is increasing evidence supporting a role for innate immune system/inflammation in the aetiology/pathophysiology of psychiatric diseases but the origin of this response remains elusive. In this regard, it is necessary to better explore two specific mechanisms possibly involved in TLR-4 signalling activation: (1) bacterial translocation of the Gram-negative enterobacteria (“leaky gut”) and (2) prenatal infection (bacteria, virus, and protozoa) induced maternal immune activation (MIA)/inflammation and the resultant oxidative/nitrosative stress.

(d) In addition to the role in the innate immune response, TLRs have an important role orchestrating adaptive immunity. However, we are still far from a complete knowledge about what is first in neuropsychiatric diseases, the unspecified TLR activation by PAMPs or DAMPs or the cytokine or chemokine signalling cascade stimulated by TLR activation. These complex series of interactions are general limitations of the current studies, and need to be addressed in the future. Although a large body of evidence illustrates that cytokines have a major impact on many brain functions under normal and pathological conditions and the potential role of cytokines in the development of neuropsychiatric diseases has already been summarized in a number of excellent reviews, there is a lack of specific studies addressing which part of the cytokine effects are TLR independent in these diseases.

(e) The identification of the elements of the TLR-4 signalling pathway in the periphery as trait/state biomarkers in CNS diseases deserves further investigation, as well as the search for hyper/hypo functional genetic polymorphisms. Their role has been explored and revised for infections and chronic inflammatory pathologies (Noreen et al., 2012), but this promising
research may be extended to neurologic/neurodegenerative and psychiatric disorders.

(f) There is increasing evidence implicating TLR-4 in the pathophysiology of psychiatric disease but the findings are still very descriptive and obtained in a reduced number of subjects. There is a need for combined translational studies using different approximations and methods, such as fMRI, brain morphometry, postmortem studies, cognitive assessments, peripheral biomarkers and genetic/epigenetic studies. These studies should investigate all the specific phases in clinical evolution of psychiatric disease because the inflammatory response is complex and TLR-4 could be implicated in some compensatory or decisive attempts against deleterious cellular events. TLR-4 agonist or antagonism could be the proper strategy depending of the state of the disease.

(g) In all future studies in humans there is a need for a strict control of the possible confounding factors that can be altering the ubiquitous TLR-4 expression and activity, with special focus on the putative effects of antidepressants, mood stabilizers and antipsychotics. Examples of these possibilities are already described for lithium or risperidone/paliperidone in animal models of neuropsychopathologies (Dong et al., 2014; MacDowell et al., 2015).

(h) To study gender differences in TLR-4-associated immune responses: women exhibit stronger cellular-mediated and humoral-mediated immune responses compared to men, and a higher risk of autoimmune diseases. Although the exact mechanisms of this difference are not fully understood, an interesting field is the study of gender differences and also the possibility to changes in female pain and pregnant women (Nicotra et al., 2012; Jang and Gilkeson 2014).

(i) To study the existence of TLR-4 SNPs hypo/hyperfunctional polymorphisms in neuropsychiatric diseases is a field that needs to be expanded. As already mentioned, a genetic association between bipolar disorder and TLR-4 SNPs has been recently found but this relationship should be checked in other neuropsychiatric pathologies. In addition, other SNPs polymorphisms of downstream elements of the TLR-4 signalling pathway such as MD-2 or MyD88 should be also studied.

(j) Go deep in the study of other TLRs in the pathophysiology of neuropsychiatric diseases: stimulation of TLR2 with hauyuronan and of TLR3 with Poly:C (a widely used animal model of MIA-induced SZ) has been identified as inhibiting neural progenitor cells proliferation in both embryonic and neonatal periods (Okun et al., 2011).

The therapeutic potential of TLR-4 signalling pathway presents an intriguing double edged profile: while innate immune system activation is a relevant element of the pathophysiology of CNS diseases, this response is also a widely conserved protective process with multiple physiological roles. Indeed, much more effort is still necessary to translate the preclinical findings to the clinical arena.

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References


expression upregulates the p-ERK expression, and protects rat brains against ischemia.


