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TOLL LIKE RECEPTOR-4: A THERAPEUTIC TARGET IN MAJOR DEPRESSIVE DISORDER

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ABSTRACT

Recent data showed that neuroinflamation plays a key role in Major Depressive Disorder (MDD). Innate immunity seem to have a major impact on this neuroinflammation via Toll like receptor 4 (TLR-4) inflammatory cascade. The aim of this review is to present and discuss evidence showing that the TLR-4 has a relevant role on the pathophysiology of MDD and that it can be a pharmacological target for new therapeutic approaches to the treatment of this disease. Pre-clinical as well as clinical studies were reviewed herein. Pre-clinical studies applied chronic mild stress (CMS) and lipopolysaccharide (LPS)-TLR-4 agonist- and demonstrated that there was an increase in TLR-4 density, inflammatory markers underlying a depressive-like behavior. Clinical studies showed that MDD patients had increased TLR-4 expression as well as other inflammatory markers (such as NF-κβ, IL-6, CRP) in blood samples. Finally we presented evidences showing that N-acetylcisteine decreased levels of TLR-4 expression as well as improving depressive symptoms in MDD patients. In spite of growing evidences for the involvement of TLR-4 in MDD there is still a lack of drugs specifically modulating the TLR-4 receptors approved in MDD. Therefore more pre-clinical and clinical studies are needed to propose effective new therapeutic aiming this inflammatory pathway.

KEYWORDS

Toll-like receptor4, Lipopolysaccharides, Major Depressive Disorder, Innate Immunity, Central Nervous System

RESUMO

Dados recentes mostraram que a neuroinflamação desempenha um papel chave na Depressão Major (DM). A imunidade inata parece ter um impacto importante sobre este neuroinflamação através da cascata inflamatória do Toll 4 (TLR-4). O objetivo desta revisão é apresentar e discutir as evidências que mostram que o TLR-4 tem um papel relevante na fisiopatologia da DM e que ele pode ser um alvo farmacológico para novas abordagens terapêuticas para o tratamento desta doença. Para tal foram revistos estudos pré-clínicos, bem como estudos clínicos. Estudos pré-clínicos aplicaram o modelo de stresse crónico leve (CMS) e lipopolissacarídeo (LPS) – um agonista do TLR-4- e demonstraram que houve um aumento na expressão de TLR-4, marcadores inflamatórios subjacentes a um comportamento depressivo. Os estudos clínicos mostraram que os pacientes com DM revelaram aumento da expressão de TLR-4, bem como a expressão de outros marcadores inflamatórios (tais como NF-κβ, IL-6, CRP) em amostras de sangue. Finalmente, apresentamos evidências de que a N-acetilcisteína diminui os níveis de expressão do TLR-4, além da melhoria dos sintomas depressivos em pacientes com DM. Apesar da crescente certeza do envolvimento de TLR-4 na DM ainda há uma falta de fármacos aprovados que modulem especificamente os receptores TLR-4 na DM. Por conseguinte, mais estudos pré-clínicos e clínicos são necessários para propor novo alvo terapêutico eficaz para esta via inflamatória.

PALAVRAS CHAVE

Toll like receptor 4, Lipopolisacarídeos, Depressão Major, Imunidade Inata, Sistema Nervoso Central

LIST OF ABBREVIATIONS

5-HT - serotonin

BDNF - Brain-derived nuclear factor

CBT - Cognitive-behavioral therapy

CMS - Chronic mild stress model

CRP - C-reactive protein

HAMD-17 - 17-item Hamilton Depression Rating Scale

IL-1 - Interleukin-1

LPS - Bacterial lipopolysaccharide

MD-2 - Myeloid differentiation protein-2

NA - noradrenaline

NF-κβ - Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B cells

NGF - nerve growth factor

OB - Olfactory Bulbectomized

RNS - reactivenitrogen species

ROS - reactive oxygen species

TNF-β - Tumor Necrosis Factor-β

HPA - hypothalamic-pituitary axis

INTRODUCTION

Mood disorders are a group of diagnoses where a disturbance in the person's mood is hypothesized to be the main underlying feature, according to the Diagnostic and Statistical Manual of Mental Disorders (DSM IV TR) classification system. These disorders are highly prevalent and deeply distress our society. However their pharmacological approach is far from being satisfactory.

There is a growing body of pre-clinical and clinical evidence suggesting an association between specific mood disorders and the innate immune system. In particular, it was recently proposed that the innate immune system can regulate brain function at the cellular and systems levels thus contributing to the pathology of mood disorders including major depressive disorder.

Toll-like Receptor (TLRs), which are single, membrane-spanning, non-catalytic receptors, play a prominent role in the innate immune system. Additionally, these receptors are usually expressed in sentinel cells such as macrophages including microglia and dendritic cells as well as in neurons. Herein we will review preclinical data supporting the role of TLR-4 signaling in the physiopathology of major depressive disorders.

Ultimately this review will highlight the TLR-4 as a new therapeutic target in major depressive disorder.

METHODS

Herein we reviewed literature indexed in Pubmed as well as Medline databases offered by both “Biblioteca Central dos Serviços de Documentação dos Hospitais da Universidade de Coimbra” and “Biblioteca do Pólo de Ciências Médicas de Coimbra”.

In the present review, preclinical and clinical studies on TLR4 as a key element on Major Depressive Disorder pathology were used as inclusion criteria. Papers which were not written in English, or which were written before 1990 were excluded.

The following keywords were used: TLR4 (toll-like receptor 4), Mood disorders, MDD, innate immunity, central nervous system, brain, LPS/lipopolysaccharide.

RESULTS

I. Epidemiology and clinical characteristics of Major Depressive Disorder

Major depression is a disorder that transcends genders, ages, races, nations, social backgrounds (1). This disorder can present itself by a large spectrum of clinical manifestations that include depressed mood, loss of drive and pleasure, loss of interest, feelings of guilt, poor concentration, low self-esteem, sleep disturbances or decreased appetit.

According to DSM-5 (2013) a person must have five or more of the following symptoms present most of the day nearly every day for at least two consecutive weeks, in order to be diagnosed with major depressive disorder. It is also mandatory that at least one of the symptoms must be either depressed mood or loss of interest or pleasure:

- Depressed mood – people with depression tend to feel sad, hopeless, discouraged, "blue," or "down in the dumps." Sometimes they do not realize they are down and instead say they feel anxious or have no feelings. Plus, some people with depression feel annoyed, frustrated, irritable, angry, or hostile;
- Loss of interest or pleasure in most or all activities (anhedonia);
- Change in appetite or weight – decreases or increases on weight are common;
- Insomnia or hypersomnia – Major depressive disorder (MDD) often disrupts sleep patterns, leading people to either sleep too much or be unable to fall asleep or stay asleep. People affected by MDD who sleep tend to describe it as not restful;
- Psychomotor agitation or retardation – this agitation can become apparent through hand-wringing, pacing, and fidgeting, whereas retardation can manifest as a slowing of body movements, thinking, or speech;
- Fatigue or loss of energy;

- Feelings of worthlessness or excessive guilt;
- Poor concentration – patients who suffer from depression are unable to think clearly, concentrating, or making decisions, are also easily distracted and can refer memory problem;
- Recurrent thoughts of death or suicide – people who are depressed can experience recurrent thoughts of death or suicide, and may attempt suicide. This "suicidal ideation," may be passive, in which case the person thinks that life is not worth living, but they can also be active, cases where the person actively wants to die or commit suicide. This is a very important sign of the severity of the pathology.

There might be psychiatric or medical comorbidities associated with MDD. Psychiatric comorbidities include anxiety disorders, posttraumatic stress disorder, obsessive-compulsive disorder, attention deficit hyperactivity disorder, substance (alcohol and drug) use disorders, just to list a few. On the other hand, medical comorbidities include general medical problems, such as diabetes, heart disease, cancer, and many others (1). There is a very complex cross-talk between MDD and the comorbidities since both can engage in positive feed-back thus contributing to worsen the overall medical condition of the patient. This can be mainly explained by the complexity of the pathophysiological mechanisms underlying MDD. This could also be partially explained because people who suffer from MDD tend to mismanage their medical conditions (2).

All this problems can lead to a variable degree of impairment in a person's capacity of taking care of themselves and/or their responsibilities. This plays a major role on the burden of the healthcare on European countries economy's (3). Major depression is the fourth leading cause of disability worldwide (4) and it is predicted that by the year of 2020 it will be the second most prevalent contributor to the global burden of disease (5). It has, in fact, a greater

decrement on health when matched against other chronic illnesses such as angina, arthritis, asthma, and diabetes (2). This is made worse when in almost a third of the depressed patients there is an absence of response or an inability to tolerate the conventional antidepressant medications (6). So, offering an alternative way of treatment of this illness calls for a rapid change in the therapeutic paradigm of MDD.

II. The role of Innate Immunity on Major Depressive Disorder

Most of the antidepressants used today modulate serotonergic and/or noradrenergic neurotransmission (7). However their effectiveness is not as high as both doctors and patients would hope. Therefore a quest for new treatment strategies lead researchers to find new evidence suggesting a key role for the innate immune system in the psychiatric and neurological diseases (7). The study of the inflammatory state in animal models or patients with MDD is especially important since it was shown that inflammatory cytokines can affect the metabolism of neurotransmitters, serotonin, norepinephrine and dopamine which has been correlated to the development of fatigue and depression (6).

Several pre-clinical investigations showed that antidepressant treatments have anti-inflammatory effects. For example, selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine and paroxetine; tricyclic antidepressants, such as imipramine; reversible inhibitors of monoamino-oxidase, such as moclobemide; "noradrenergic" antidepressants, such as reboxetine; lithium and even atypical antidepressants, such as tianeptine; all have anti-inflammatory effects by decreasing the production of IL-1β, TNF-α, IFN-γ and/or increasing anti-inflammatory cytokine IL-10 or modulating the ratio between Th1 and Th2 produced cytokines (8). It was shown in a clinical study that the efficiency evidenced by antidepressants might be enhanced by simultaneous administration of anti-inflammatory agents like celecoxib, a cyclooxygenase-2 inhibitor (9).

The innate immune system is inherently encoded to stereotypically respond to precise signals originated from pathogens or other menace signs contrary to the more evolved adaptive immune system which has the capacity to identify and remember specific pathogens which leads to a higher responsive capacity each time this same pathogen is encountered (7). Such pathogens stimulate signal transduction cascades that culminate with the release of pro-inflammatory cytokines and chemokines, the most well studied being the cytokines

interleukin 1beta (IL-β), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α). This inflammatory mediators are released by macrophages, other peripheral immune cells, and microglia on the early acute phase reaction.

Comparisons between medically ill and healthy patients, both suffering from major depression, revealed that cardinal features of inflammation were present in the two groups. In cases where depression occurs in a context of clinical illness (infectious, autoimmune, inflammatory component or tissue damage) it is easy to establish the source of infection. Nevertheless, in medically healthy patients that suffer from MDD, evidences of inflammation are abundant but the source of this inflammatory state is not as clear as it is with patients that are medically ill (10). These inflammation features comprised elevation on peripheral blood and cerebrospinal fluid of inflammatory cytokines and their soluble receptors, elevation on peripheral blood of acute phase proteins, chemokines, adhesion molecules and prostaglandins (10). Also, when administrated with cytokines and cytokines inducers such as lipopolysaccharide (LPS) – a constituent of the outer layer of gram-negative bacteria- the host has been show to developed behavioural changes (depressed mood, anxiety, anorexia, fatigue, psychomotor retardation, impaired sleep and cognitive dysfunction) that are similar to those seen in depressed patients (6). Lastly, it has been shown that the administration of drugs that inhibit inflammatory mediators or their signaling pathways leads to an improvement of inflammatory disorders patient's mood and to an augmented responsiveness to antidepressants in patients with major depression (6, 11). Also of note is the discovering of data showing that use of conventional antidepressant drugs has revealed a decrease on the inflammatory markers on depressed patients after a successful therapy (10), as well as experiments results that exposed that antidepressant drugs can inhibit inflammatory cytokines production in-vitro (12).

III. TLR: molecular structure, cell distribution and functions on the Central Nervous System

Toll-like receptors (TLRs) are type-I transmembrane receptors which play an enormous part as agents of the innate immune response. They constitute the first line of defense against invading microorganisms (13). TLRs recognize a broad range of pathogen-associated molecular patterns (PAMPs) - including bacterial cell wall components, bacterial genome DNA and viral, fungal and parasitic products - and host-derived damage-associated molecular patterns (DAMPs), which can also be designated as "endogenous ligands", produced in situations of traumatic tissue injury, inflammation and stress. The TLR family has eleven functional members in mammals (TLR-1 – TLR-11) (14). They can be divided into two large subgroups based on their cellular distribution. While the TLR-3, TLR-7, TLR-8, TLR-9 and TLR-10 are expressed exclusively in the intracellular compartment, the TLR-1, TLR-2, TLR-4, TLR-5, TLR-6 and TLR-11 are cell surface molecules. TLRs are expressed in numerous cell types in the central nervous system, including microglia (TLR1-9), astrocytes (TLR1-5;TLR-9) and neurons (TLR-3) (15).

The binding of DAMPs or PAMPs to TLRs results in the activation of signaling cascades that involve the transcription factors nuclear factor for the kappa light chain enhancer in B cells (NF-κβ) and activating protein-1 (AP-1), which result in the production of inflammatory cytokines/chemokines such as TNF-α, IL-1 beta, IL-6, IL-8, and IL-12 (15, 16).

Further roles have been associated with TLRs other than their crucial inflammatory role: this receptors are intimately involved in the neurogenesis, learning and memory in the absence of any underlying inflammation/infectious aetiology (17).

IV. TLR-4and Major Depressive Disorder

Several factors that provoke a low-grade inflammation and raise the risk of MDD have been recently reviewed (18). These MDD risk factors include the following: psychosocial stressors (acute psychological trauma or more sub-chronic stressors, and early exposure to childhood trauma), "Western-type" diet which contains highly refined carbohydrates and saturated fatty acids, low levels of exercise, smoking, obesity and deficiencies in vitamin D. Stress-induced activation of cytokine responses in the CNS is largely dependent on the activation of microglia, where TLR-4 is present (19). Another mechanism that has been identified as a relevant player in MDD-associated inflammation is increased gut permeability, which leads to an increased number of bacteries in the systemic circulation with a resulting trigger of the TLR-4 pathway (20).

Moreover, a recent meta-analysis showed that anti-inflammatory drugs might be effective in the treatment of mood disorders which would be an aspect in favour of the hypothesis that inflammation has a causal role in this pathologies (21).

TLR-4 is a widely studied TLR within pro-inflammatory settings, is activated by bacterial lipopolysaccharide (LPS) and responds predominantly through its co-receptor, myeloid differentiation protein-2 (MD-2) (22).

Ligand binding fosters the formation of a TLR4 complex with CD14 (23). TLR-4 co-receptor, MD-2, contributes to this complex by coupling with the TLR4 extracellular domain that recognizes bacterial LPS thus triggering a network of intracellular cascade in which the transcription factor NF-κβ plays a key role by facilitating the expression of pro-inflammatory cytokines, such an interleukin-1 (IL-1) and Tumor Necrosis Factor-β (TNF-β), and facilitating the synthesis of arachidonic-acid derivatives (18).Type 1 interferons are also increased following TLR4 activation.TLR4 signaling evokes macrophages, natural killer cells, mast cells, and others attraction, leading to the release of reactive oxygen species (ROS) and

reactive nitrogen species (ROS) (fig. 1). This increased production of ROS contributes to the first line defence by killing the invading microbiological pathogen (24). On the other side, the release of ROS after "sterile infections," in which DAMPs activate TLRs, could be more harmful because the radicals may damage the host's tissues.

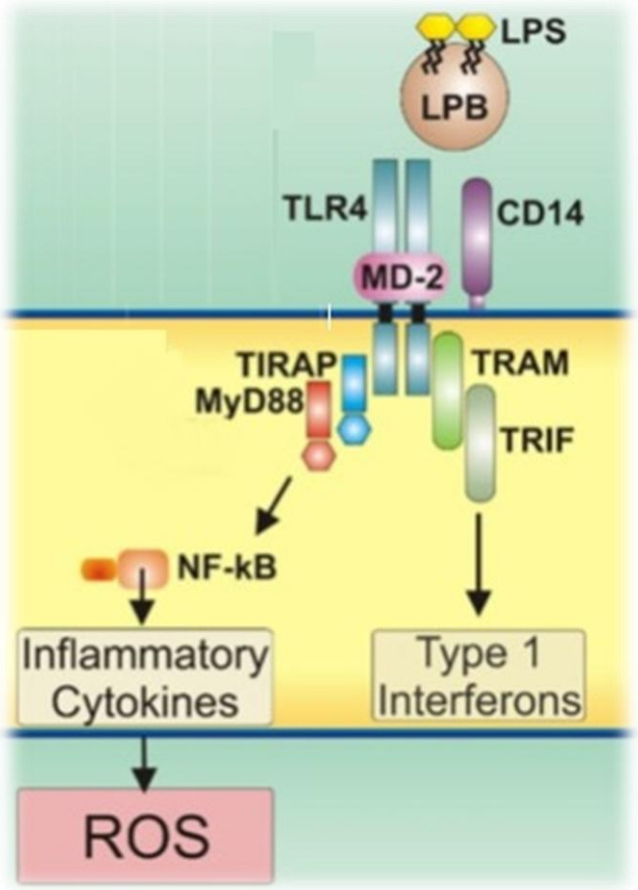

Fig. 1 Toll-like receptor (TLR) activation. Toll-like receptor 4 are part of the innate immune system. TLR4 recognize lipopolysaccharides (LPS) and its activation occurs through receptor dimerization. TLR4 builds homodimers. TLR4 activation ensues when LPS binds to lipopolysaccharide-binding protein (LPB). Cluster of differentiation 14 (CD14) and myeloid differentiation factor-2 (MD-2) are required for TLR4 dimerization. TLR4 signaling can follow two different intracellular pathways. The MyD88-dependent pathway via TIRAP induces the transcription factor nuclear factor-κB (NF-κB) resulting in the release of inflammatory cytokines, e.g., interleukin 6 and tumor necrosis factor-α. Enhanced amounts of reactive oxygen species (ROS) will be produced. Alternatively, the MyD88-independent pathway via TRAM and TRAF leads to the release of type 1 interferons. Adapted from Lucas and Maes (24).

There are two main mechanism proposed to activate brain TLR-4: one is related to DAMP/PAMP released from disrupted cells and extracellular matrix degradation products that may contribute to immune activation after brain injury (25). The other one is known as "leaky gut" and was revealed after some models of stress showing an increase in intestinal permeability and, as a result, a bacterial translocation to the systemic circulation (26). These Gram-negative Enterobacteriaceae are, obviously, a significant source of LPS, which leads to an activation of brain TLR-4 pathways, therefore enhancing a neuroinflammatory response. Under normal circumstances, Gram-negative bacteria are isolated from the lymphatic system and systematic circulation by tight junctions between the epithelial cells of the gut. If this barrier is weakened (''leaky gut''), Gram-negative bacteria will interact with the immune system producing an abnormal response in MDD (4,27,28). After developing a new animal model of depression that tested the ''leaky gut'' mechanism it was shown that chronic mild stress amplified bacterial LPS in the circulation of rats and a subsequent activation of the TLR-4 pathway that may be, at least in part, responsible for behavioral despair in animals. Along this lines antibiotic intestinal decontamination prevents stress-induced elevation of LPS binding protein in the circulation and TLR-4 activation in the frontal cortex of experimental animals (26). However, some evidence suggests that the loss of intestinal barrier is not sufficient to initiate MDD and there are no studies revealing that the restoration of this barrier can lead to clinical improvement (29).

V. TLR-4 role on MDD: Pre-clinical studies

Chronic mild stress model (CMS) is an external stress-induced animal model of depression which was used to study the hypothesis that an external stressor might be the cause of systemic inflammation, thus triggering neuroinflammation, neurotoxicity, decreased neurogenesis and depressive-like behaviour (30). The authors also attempted to disclose the mechanisms underlying anti-depressants effects in this model.

These authors argued that external stress leads to an augmented expression of pro-inflammatory cytokines and activation of various inflammation-related pathways in the CNS. In particular, external stress models of depression showed that depression-like behaviors - long-lasting changes of locomotor activity, weight loss, altered diurnal rhythms, sleep disturbances and anhedonia-were accompanied by increased IL-1β, IL-6 and TNFα levels while also inducing increases in NFκB, COX-2, TLR4 expression and oxidative stress, including lipid peroxidation. External stress also elicited neuronal cell damage, apoptosis (increased caspase-3 and lowered expression of Bcl-2) and a marked decrease in neurogenesis in the hippocampus.

Regarding the administration of antidepressants (desipramine) it was shown that these drugs weaken the depressive-like behaviour and the (neuro)inflammatory phenotype. On one hand the anti-inflammatory effects are credited to the direct effects of antidepressants in pro-inflammatory cytokines (IL-β, TNF-α, IL-6) and to their stimulatory effects on anti-inflammatory cytokines (IL-10). On the other hand the neurogenic/regenerative effects are credited to stimulatory effects on the expression of different neurotrophic factors, in particular trkB, the receptor for brain-derived nuclear factor (BDNF), and down-regulation of apoptotic pathways by activating Bcl-2 and Bcl-xl proteins, and suppressing caspase-3 and -8 and Bax.

Another animal model of depression used **LPS** intravenously or intraperitoneally and it was showed that LPS administered is capable of causing depression-like symptoms (reduced exploratory behaviour, reduced social interaction, reduced libido and increased anhedonia) (31).

This is especially important since it is well documented that LPS plays a major role in the activation of the TLR-4 signaling pathway. Therefore this model just adds to the crescent notion that TLR-4 activation by LPS is a key element to the pathophysiology of MDD. It is also what made possible the appearance of the "leaky gut" hypothesis. However, "leaky gut" role in MDD is not fully understood because a "low-grade" inflammatory state leads to the migration of enteral bacteria to systemic circulation, which in turn will have an upregulating effect on the TLR-4 signaling pathway that in turn contributes, itself, to an inflammatory state. Hence the difficulty of understanding if "leaky gut" is just another mechanism that contributes to the inflammatory state on MDD patients or if it can, actually, be the cause of some cases of MDD. Some evidence suggests that the loss of intestinal barrier is not sufficient to initiate MDD and there are no studies revealing that the restoration of this barrier can lead to clinical improvement (29). However that does not make the "leaky gut" hypothesis any less relevant, in fact it may even just highlight that much more attention should be paid to it. Keri *et al.* (18) demonstrated intestinal decontamination down-regulates several components of LPS-induced neuroinflammation but it is not able to wholly avert the behavioural effect of chronic mild stress. This is where TLR-4 signaling pathway downregulation might be of critical use: an association between intestinal decontamination and TLR-4 antagonist. Studies should be promoted in order to understand if it is possible that this association can be effective in MDD treatment.

VI. TLR-4 role on MDD: Clinical studies

Keri *et al.(18)* investigated TLR-4 mechanisms in newly diagnosed, drug-free patients experiencing their first lifetime major depressive episode. Also, these assessments were repeated after a complete series of sessions of cognitive-behavioral therapy (CBT) to achieve an improved understanding of how behavioral and cognitive interventions affect inflammatory mechanisms in MDD. In order to do this the expression of TLR-4 at the mRNA and receptor protein level were evaluated in peripheral blood mononuclear cells, along with the expression of NF-κB. As it is not activated by Gram-negative bacteria, TLR-2 served as a control molecule. The low-grade non-specific systemic inflammation was assessed by the measurement IL-6 and C-reactive protein (CRP) in serum. The "leaky gut" hypothesis of MDD was tested by investigating serum markers associated with gut barrier permeability and bacterial translocation.

The results showed an up-regulation of the TLR-4 signaling pathway in these newly diagnosed and untreated patients. This was revealed by an increased expression of TLR-4 RNA and protein along with NF-κβ. When markers associated with gut barrier permeability and bacterial translocation aroused so did TLR-4 RNA/protein and NF-κB levels. CBT decreased the expression of TLR-4 and NF-κβ, which exhibited a positive correlation with depressive symptoms improvement. There was no elevation on the expression of TLR-2 in MDD which allows the inference that the innate immunity is to some extent specific in this pathology since TLR-4 is stimulated by molecular patterns of Gram-negative and TLR-2 is stimulated by molecular patterns of Gram-positive.

Hung *et al*. (15) investigated whether TLRs expression levels were altered in patients with MDD and how these changes correlated with the severity of symptoms. Therefore 30

patients with MDD and 29 healthy controls were enrolled in the study. The patients with MDD were evaluated using the Structured Clinical Interview for DSM-IV Axis I disorders and the severity of the depression was assed using the 17-item Hamilton Depression Rating Scale (HAMD-17). The mRNA expression levels of TLRs were examined in parallel with a housekeeping gene in peripheral blood using real-time polymerase chain reaction (RT-PCR). These authors showed that TLR4 mRNA levels were significantly higher in individuals diagnosed with major depressive disorder than in healthy controls. These findings give evidence to the importance of TLRs expression level changes in patients with major depressive disorder. This TLR-4 mRNA overexpression is compatible with the results from previous animal experiments. It was also shown N-acetylcistein and melatonin regiment, that reduce levels of TLR-4 mRNA or protein expression can in fact improve chronic mild stress-induced behavioural dysfunctions in mice or depressive symptoms in human (32).

Jones and Thomsen (7) investigated the manifestations of the pro-inflammatory state in depression-like states. This helps to make the connection between an inflammatory state tightly linked to the TLR-4 signaling pathway like that of a MDD patient and its symptoms. Pro-inflammatory state in depression-like states has a wide range of presentations. It might appear as periods of anxiety and memory deficits in normal volunteers subjected to low doses of bacterial pathogen LPS or attenuated rubella virus (33), higher incidence of depression in individuals with an history of myocardial infarction with an consequent elevated level of inflammatory markers (34) or as an increased prevalence of depression in patients suffering from severe rheumatoid arthritis (35).

VII. TLR-4 signaling pathway: a putative target for MDD management?

TLR-4 signaling pathway is especially important in inflammatory context since it offers several opportunities for pharmacological interventions. The following are the most studied approaches concerning the modulation of the TLR-4 signaling pathway:

1. Anti-LPS designed to neutralize LPS
2. TLR-4 antagonism, including the MyD88 signaling pathway
3. Targeting ROS

Neutralizing LPS

Since this is a promising therapeutic approach many synthetic anti-LPS peptides (SALPs) have been developed (OxPAPC and LPS-RS are already available for application in research). They bind LPS preventing the activation of TLR-4 complex. This has been demonstrated in a mouse model of lethal sepsis where these drugs were able to neutralize bacterial endotoxins even at minimal levels and safeguard the animal from endotoxic shock. Although SALPs seem likely a very promising tool for the treatment of MDD there are no preclinical studies testing this hypothesis. Moreover this strategy would pursue the leaky-gut hypothesis.

TLR-4 Antagonism

Herein we will review representative TLR-4 antagonists.

Eritoran is a synthetic lipid A that connects to MD-2 and prevents LPS activation of TLR4, being of relevant use in the treatment of different inflammatory diseases and is currently in a phase III study in sepsis.

A LPS-like molecule named **cyanobacterial product** (CyP), extracted from the *cyanobacterium Oscillatoria Planktothrix*, does not activate TLR, but successfully binds MD-2. As MD-2 is vital for TLR4 activation by LPS, this lipid prevents LPS-mediated inflammatory responses by occupying the co-factor MD-2. CyP protected mice from endotoxin shock (36).

Another option to inhibit the activation of TLRs is the use of specific monoclonal antibodies that might neutralize human TLR4- induced cellular activation.

Surprisingly, diverse plant compounds may be used as antagonists to target the TLR4 complex, as evidenced by the efficacy of many herbs used in Traditional Chinese medicine and Ayurveda medicine. Green tea has a component (**epigallocatechin-3-gallate**) that is an inhibitor of kinase TBK1 and can suppress the MyD88 signal and the MyD88-independent signal pathway. Ginger contains **6-shoagol** which inhibits inhibitor-κβ kinase. **Curcumin** antagonizes NF-κβ activation while also inhibiting the dimerization of TLR-4 (24).

MyD88 is another important target in the innate immune system. One substance that can suppress the induced overexpression of MyD88 in vivo and in vitro is **cinnamon extract** in high concentrations. In studies it was shown that cinnamon extract may prevent alcohol-induced liver damage in mice. In vitro studies discovered that cinnamon extract suppresses TNF-α expression and nitric oxide (NO) synthesis as well as LPS-induced MyD88 and iNOS. **EM-163** is a synthetic mimetic of MyD88 that acts by aiming at the TIR domain, which averts downstream MyD88 signaling. EM-163 inhibits the production of proinflammatory cytokines in human primary cells, such as IL-1, IL-6, TNF-α, and IFNγ. Morever **propofol**, an anesthetic, has been shown to have the ability to reduce ROS formation, suppress NF-κβ expression and reduce IL-6, probably acting upstream of NF-κβ but its mechanism is not quite cleared up until now. A recent clinical study suggested that propofol may improve MDD in patients who are receiving ECT (37).

Blocking ROS

TLR-4 inflammatory cascade leads to augmented production of ROS. Radicals are a double-edged sword in the body. While ROS released during inflammatory responses inactivate bacteria, viruses, and fungi, ROS may damage its own tissues during sterile inflammation where no pathogens are present.

Several substances can be used allowing the elimination of ROS while modulating the TLR comlplex: synthetic substances like **N-acetylcisteine** and natural substances as alfa-lipoic acid and some flavonoids. N-acetylcisteine can reduce levels of TLR-4 mRNA or protein expression which leads to an improvement on behavioral dysfunctions induced by chronic mild stress and depressive symptoms in humans (32, 38). **Alfa-lipoic acid** (ALA) showed decreased levels of TLR-4, HMGB1,IL-6, TNF-α and inflammation markers when compared with the control group, in a LPS model. Two substances **glycyrrhizin** (GL) and **isoliquiritigenin** (ILG) from the plant *G. uralensis* (Chinese licorice) suppress LPS-induced TLR4 signaling in different ways. Whereas GL prevents the formation of the LPS–TLR4/MD-2 complex, which leads to an inhibition of homodimerization of TLR4, ILG inhibits LPS-induced TLR4 homodimerization.

CONCLUDING REMARKS

This review clearly highlights the role of the TLR-4 pathway on MDD. While this pathway has not yet offered a specific drug therapy for MDD, it stresses the need for future pre-clinical as well as clinical studies on the promising field of innate immunity mood disorders.

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