Review paper

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Does lipopolysaccharide-based neuroinflammation induce microglia polarization?

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Abstract

Lipopolysaccharide (LPS) is a potent immunogen when administered locally and/or systemically. The peripheral immunization with LPS could contribute to the progression of neurological diseases because a strong link between neuroinflammation and dopaminergic degeneration has been found. The switch between the survival and neuronal death in substantia nigra could be related to M1 (neurotoxic) and M2 (neuroprotective) microglia phenotypes. In this review, we present the current findings about microglia roles, biomarkers, and natural or synthetic immune modulators determined in the LPS-based murine model.

Key words: *cell signalling, lipopolysaccharide, proliferation, microglia cells, neuroinflammation.*

Introduction

Neuroinflammation is defined as an inflammatory response mediated by the production of cytokines (tumour necrosis factor α (TNF-α), interleukin (IL)-1β, IL-4, IL-6), chemokines, mitochondrial nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, inducible nitric oxide synthase (iNOS), reactive oxygen species (ROS, superoxide, hydroxyl radicals); and gliosis (astrocytes and microglia) in the central nervous system (CNS) and in the peripheral nervous system (PNS) [7,24,25,55]. In this context, the microglia activation has been associated with persistent neuronal damage, changes in long-term potentiation, motor and cognitive dysfunction in neurodegenerative diseases [9,37,87,96]. But as per the recent studies, microglia is related to maintenance of homeostasis, modulation of neurogenesis and cognitive processes in the healthy adult brain [3].

To understand the complete cellular and molecular mechanisms underpinning the neuroinflammatory diseases such as Parkinson disease (PD), paraquat, 6-hydroxydopamine (6-OHDA) and lipopolysaccharide (LPS) induced animal models have been used. The paraquat model enables the degeneration of dopaminergic and gamma aminobutyric acid (GABAergic) neurons with a slight presence of inflammation; while the 6-OHDA model, although

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it promotes the death of dopaminergic neurons, mainly in the *substantia nigra* (SN), is accompanied by discrete neuroinflammation at the site of injury [11,42,74,90].

Lipopolysaccharide is an endotoxin found in the cell wall of gram-negative bacteria, which can interact with a variety of cells through the toll-like receptor 4 (TLR4) [64,69]. The local or peripheral administration of LPS activates microglia [104] and astrocytes [46], immune cells and macrophages-like cells in different organs of the body [55,56,83]. The peripheral injection of LPS-induced endotoxemia favours increased brain levels of interleukins, prostaglandins, nitric oxide, and other modulator substances. Injection of LPS directly into the CNS can induce an inflammatory response similar to peripheral administration [29,38,81,85]. Hence, the pathophysiology of PD, neurological disorders related to prenatal systemic inflammation, septic shock, gut-brain axis in maintaining homeostasis [22] and the search for new anti-inflammatory drugs can also be studied using LPS-induced models [4,5,34,39].

Lipopolysaccharide-related murine models

In the central and peripheral nervous systems, the LPS effects have been evaluated from several doses and two administration routes (local and peripheral). Two to ten µg of LPS has been used for unilateral intracranial injection [4,8,44,94], while doses of 0.05 to 5 mg/kg have been evaluated in models of intraperitoneal injection [13,17,87], highlighting the LPS derived from *Escherichia coli*, serotype 055:B5 as the most used one in preclinical research. With regard to main LPS effects, the intracranial injection of this immunogen produces dose-dependent microglial activation and increases neurodegeneration in susceptible brain nuclei (e.g. SN) in mice and rats [4,8,30,44,55,56,93]. Furthermore, depending on the LPS that is injected in SN [24,78], globus pallidus [102], neostriatum [19] or lateral ventricles [43], the microglial activation is indistinctly characterized by morphological changes, cellular stress and anti- and pro-inflammatory cytokine production. On the contrary, the intraperitoneal injection of LPS in adult rats (LPS-induced endotoxemia) causes microglia migration (and priming) in a dose-dependent manner in *substantia nigra* (*pars compacta*, *compacta dorsal* and *pars reticulata*) and damage in peripheral organs [10,13,17,87]; while in mice the chronic LPS administration induces motor deficits caused by alterations in the dopaminergic neurons of SN [16,50,65], and cellular stress in organs [72]. In addition, LPS promotes loss of dopaminergic neurons of neonatal rats when their mothers receive the intraperitoneal administration of this immunogen on day 9.5 [39] or 10.5 prenatal [110].

Lipopolysaccharide and microglia polarization

Lipopolysaccharide has been used as a classical toxin to activate immunocompetent cells and trigger a systemic and central inflammatory response [56,96]. LPS consists of a lipid portion called lipid A, a lipopolysaccharide O (core) and an oligosaccharide O. Lipid A is responsible for the activation of macrophages and the production of pro-inflammatory cytokines. The polysaccharide O gives the bacteria its serological specificity, and the oligosaccharide O is responsible for linking the polysaccharide O to the lipid A [84]. The LPS binds to the specific receptor complex CD14/TLR4/LBP that is found in the membrane of microglial cells and induces their activation. The LPS, once recognized by the membrane-associated CD14/TLR4/LBP receptor complex, transports the lipid A to the MD-2 protein, which can be found soluble or bound to TLR4 through hydrogen bonds [1,54]. The formation of this complex is the key for the initiation of intracellular signalling.

Microglia are the resident mononuclear phagocytes in the CNS that belong to the non-neuronal glial cells [36]. Microglia change their morphology on activation from the inactive or resting state due to the CNS damage caused by infections, brain trauma, injury, ischemia, etc. The activated microglia exist either in the classical M1 phenotype or alternative M2 phenotype and these two phenotypes are the two activated states that represent their extreme biological functional states and determine their physiological characteristics. The activated classical M1 phenotype is seen due to the presence of high levels of interferon (IFN), tumour necrosis factor (TNF) and LPS and in turn produces and releases inflammatory mediators that are toxic to the neurons, thus leading to the neuroinflammation or neurodegeneration and damage [26]. On the contrary, the activated alternative M2 phenotype is seen due to the presence of high levels of interleukins (IL-4, IL-13) and in turn produces and releases anti-inflammatory cytokines and

various neurotrophic factors that protect and regenerate the neurons, thus leading to the neuroprotection and recovery [61].

As per the recent studies, M1 and M2 phenotypes can be distinguished basing on inflammation-related cytokines [48], specific cell membrane proteins and arginine metabolism-associated molecules [97]. LPS is a TLR4 agonist that can polarize the microglia into the M1 phenotype and also effectively induces inflammatory responses, thus reducing the M2 phenotype and its anti-inflammatory markers [59]. This is the reason behind selecting LPS-induced neuroinflammation in various studies. LPS-induced neuroinflammation is mediated by TLR4/nuclear factor (NF)-κB signalling pathway and the expression of NF-κB is crucial for the M1 phenotype of microglia [103]. In order to cure neuroinflammation, several researchers have proposed different sources but the common solution among all that research includes the inhibition of the M1 phenotype and promotion of the M2 phenotype by suppressing TLR4/NF-κB pathways [79,101,105]. Even though several models exist for neuroinflammation, LPS is the best and most widely used one [6], especially as those including the study of microglia as LPS play a key role in microglia polarization.

In vitro and *in vivo* studies have suggested that LPS exerts microglia polarization as evidence of microglia plasticity [68,91]. For example, the unilateral microinjection of 5 µg of LPS in SNpc of male rats induces an increase in the M1 phenotype, which was associated with the loss of dopaminergic neurons at 1, 3 and 7 days post-injection. This neurotoxic event was evidenced by high levels of oxidative stress, upregulated expression of iNOS, cyclooxygenase 2 (COX-2) and nitrotyrosine, and downregulated expression of arginase 1. However, facts were reversed with capsaicin, where a neuroprotective effect, characterized by the M2 microglial phenotype, is increased, arginase 1 expression is increased and low levels of oxidative stress markers in rat SN was determined [8]. In the female rats, the single administration of 5 µg of LPS into SNpc favours loss of dopaminergic neurons at 7 days after microinjection. In the first 24 h post injection of LPS, a neurotoxic effect was evidenced by M1 microglial activation, accumulation of fluorescent oxidized hydroethidine, upregulated proinflammatory markers (NF-κB p-p65), inflammatory markers (TNF-α, IL-1β and PEG2) and oxidative stress markers (COX-2 and iNOS). However, this fact was reversed with pre-treatment by immune modulators such as somatostatin, where an increased level of M2 polarization and low levels of neuronal death or oxidative stress was observed [4]. Additionally, the conversion of M1 to M2 polarization is determined using treatments with dexamethasone [12], Ginsenoside Rg1 [88], G1 agonists [62] or polydatin [44].

A lot of evidence has been used to support that LPS favours the M1 microglia phenotype, because of increased CD86, IL-1β, CCL5, IL-6, iNOS, and CXCL1 mRNA levels, but it did not induce significant changes in Arg1, CD06, IGF-1 and PPARg mRNA levels, which are microglia phenotype markers [89]. Some studies suggested that such factors as dosage and combination of LPS with other stressors can alter the M1/M2 switch [17,80]. In this context, a study performed by Rabenstein *et al*. [73] reported that the microglia polarization is a derivative of LPS, or LPS with a cell pre-conditioned by ischemia and in a primary microglia culture obtained from pup rats; in the 10 ng/ml LPS group, the polarization to the M1 microglial phenotype was detected, while that in the group with LPS and pre-conditioning level increased of IGF1, an anti-inflammatory factor [40], was found. Serdar *et al*. [76] injected intraperitoneally 0.1 mg/kg LPS followed by hypoxic ischemic brain injury in rats; this study demonstrated reduction in neuronal density, microglial activation on hippocampus and cerebral cortex, changes in gene expression profiles associated with M1/M2 microglial polarization. They observed a significant increase in the gene expression levels of *iNOS*, *IL-1* and *IL-6* in the LPS group. However, in the LPS/hypoxic ischemic brain injury group, the increase in these molecules was more significant, showing that even if LPS is a powerful microglial activator, the polarization of these cells is closely related to the simultaneous events that occur in the body.

M1 and M2 microglia, cell signalling and modulators

The level of activation of microglial cells depends on the intensity, duration and type of the stimulus and can generate a neuroprotective or neurotoxic response [30,31]. At the level of the CNS and PNS, two phenotypes of microglia, M1 and M2, have been distinguished. The M1 phenotype (neurotoxic) is characterized by the release of pro-inflammatory factors from thin branched microglia that can be

derived to amoeboid (typical hallmark of neurodegeneration); while M2 microglia retains branched morphology and promotes the release of anti-inflammatory factors leading to neuroprotection [8,92] (Fig. 1). These morphological changes have been associated with the expression of the genes *Ccr2*, *Nox2*, *TNF*, *Ly6c*, *IL12a*, *IL12b* and *Icam1* [17]. To assess microglial activation, markers such as ionized calcium binding adapter molecule-1 (Iba-1) [44], cluster of differentiation 68 (CD68) [67], CD11b or CD11b/c [8], CD206 [2] and complement receptor type 3 (CR3) [109], which is commonly named as OX42 [30], have been used. Among these markers, the most common ones are CD16/32 and CD86 for the M1 subtype, and CD206 and CD11b for M2 microglia [108] (Fig. 2).

The M1 and M2 microglia phenotypes exhibit different intracellular signalling. In Figure 2, the interaction of anti- or pro-inflammatory mediators to define microglia roles is illustrated. In the case of M1 microglia, the binding of LPS to the MD2/TLR4 induces the upregulation of *nuclear factor kappa-light chain enhancer of activated B-cell* (*NF-κB*) gene, *via* myeloid differentiation primary response 88 (MyD88) dependent pathway, leading to the release of the first phase of pro-inflammatory mediators (PIMs) such as TNF-α, IL-1α, IL-1β, IL-6, IL-12, IL-18 and IL-23 and *interferon regulatory transcription factor 3* (*IRF3*) gene, *via* MyD88 independent pathway, leading to the release of the second phase of PIMs such as IFN-α, IFN-β, IFN-γ, IFN-ω, IFN-ε, IFN-κ and IFN-ζ. The released PIMs such

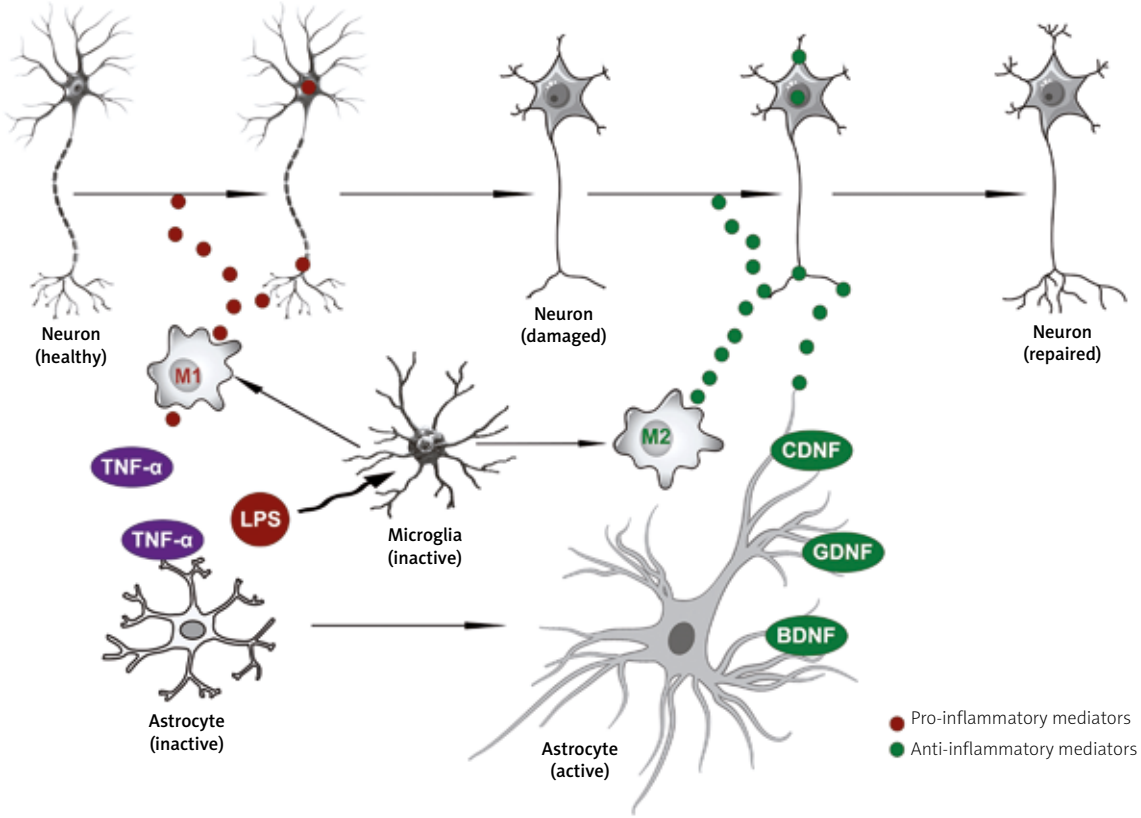
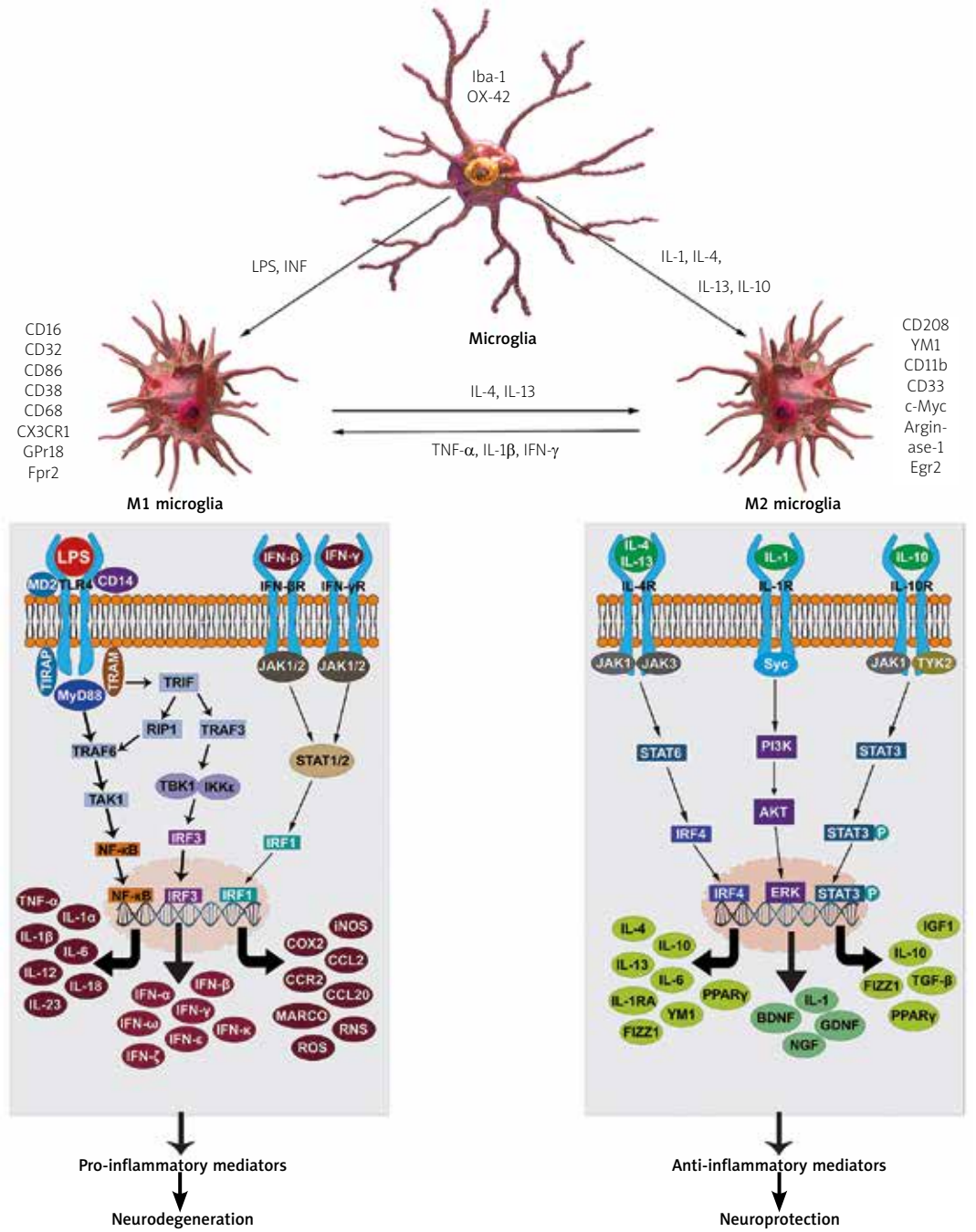


Fig. 1. Switch between neuroprotective neurotoxic microglia in an lipopolysaccharide (LPS)-related model. LPS induces the activation and conversion of inactive microglia to active M1 neurotoxic microglia, which in turn releases pro-inflammatory cytokines that are taken up by a healthy neuron in a retrograde manner and thus causes neuronal damage. The production and release of TNF-α, also a pro-inflammatory cytokine released by microglia, induces the activation of astrocytes. Thus, activated astrocytes release large amounts of anti-inflammatory cytokines and certain neurotrophic factors, such as BDNF, CDNF and GDNF, which in turn induce the conversion of inactive microglial cells to active M2 neuroprotective microglial cells. Thus, activated microglial cells also release neurotrophic factors and anti-inflammatory cytokines that are taken up by damaged or degenerated neurons and leads to the recovery of the neuron from damage induced by LPS.



AKT – activator of protein kinase B, BDNF – brain derived neurotrophic factor, CCL-c – c chemokine ligand, CCR-c – c chemokine receptor, CD – cluster of differentiation, COX – cyclooxygenase, CX3CR1 – C-X3-C motif chemokine receptor-1, EGR – early growth response, ERK – extracellular receptor kinase, GDNF – glial cell-line derived neurotrophic factor, GPR – G-protein coupled receptor, Iba – ionized calcium-binding adaptor protein, IFN – interferon, IGF – insulin-like growth factor, IKK – I kappa B kinase, IL – interleukin, iNOS – inducible nitric oxide synthase, IRF – interferon regulatory transcription factor, JAK – Janus kinase, LPS – lipopolysaccharide, MARCO – macrophage receptor with collagenous structure, MyD – myeloid differentiation factor, NGF – nerve growth factor, NF – nuclear factor, OX-42 – an antibody designed to detect CD11b, PPAR – peroxisome proliferator-activated receptor, RIP – receptor interacting protein, RNS – reactive nitrogen species, ROS – reactive oxygen species, STAT – signal transducer and activator of transcription, TAK – TGF-β activated kinase-1, TBK – Tank binding kinase-1, TGF – transforming growth factor, TIR – toll interleukin-1 receptor, TIRAP – TIR domain containing adaptive protein, TLR – toll-like receptor, TNF – tumour necrosis factor, TRAF – TNF receptor associated factor, TRIF – TIR domain containing adaptor-inducing interferon β, YM-1 – neutrophil granule protein.

Fig. 2. Schematic view of lipopolysaccharide (LPS)-related cell signalling in M1 and M2 microglia subtypes. Presence of LPS or interferon (IFN) induces the activation of microglia (M1 type). Binding of LPS to toll-like receptors (TLR) and IFN-β and IFN-γ to their corresponding receptors (IFN-βR and IFN-γR) leads to the induction of NF-κB, IRF3 and IRF1 genes through corresponding pathways. These genes in turn lead to the production and release of huge amounts of pro-inflammatory mediators, which eventually results in the neurodegeneration. Similarly, the presence of interleukins (IL)-1, IL-4, IL-10 and IL-13 induces the activation of microglia (M2 type). Binding of IL-4, IL-13 to IL-4R, and IL-1 and IL-10 to their corresponding receptors (IL-1R and IL-10R) leads to the induction of IRF4, ERK and STAT3 genes through their corresponding pathways. These genes in turn lead to the production and release of huge amounts of anti-inflammatory mediators, which eventually results in the neuroprotection. The presence of IL-4 and IL-13 in huge amounts also switches the M1 microglia to M2 microglia directing towards neuroprotection. Whereas, the presence of TNF-α, IL-1β and IFN-γ in huge amounts switches M2 microglia to M1 microglia and directs towards the neurodegeneration. All the markers for microglia, M1 microglia and M2 microglia are mentioned next to the image in the figure.

as IFN-β and IFN-γ induce the upregulation of *IRF-1 via* Janus kinase (JAK) – signal transducer and activator of transcription (STAT) pathway, leading to the release of the third/final phase of PIMs such as COX-2, chemokine ligand 2 (CCL2), c-c chemokine receptor 2 (CCR2), CCL20, MARCO, reactive oxygen species (ROS), reactive nitrogen species (RNS) and iNOS (inducible nitric oxide synthase) [17,33,35, 63,94,95].

For the M2 microglia phenotype (Fig. 2), the binding of IL-4 and IL-13 to their receptors leads to the upregulation of *interferon regulatory factor 4* (*IRF4*) gene and releases the first phase of anti-inflammatory cytokines, mediated by JAK1/3-STAT6 pathway. A second route is related to IL-1; the binding of IL-1 to its receptor leads to the induction of *extracellular signal-regulated kinase* (*ERK*) gene and releases several neurotrophic factors, mediated by protein kinase B (AKT). Similarly, the binding of IL-10 to its receptor leads to the expression of *STAT3* gene and releases the third phase of anti-inflammatory mediators, mediated by JAK1/tyrosine kinase 2 (TYK2)-STAT3 pathway [21,32,51].

To date, several immune modulators that help in switching the microglial phenotype from M1 to M2 have been reported. Almost all these modulators, whether natural or other, decrease the markers and/or pro-inflammatory mediator production and/or release and thus direct towards the M2 phenotype for protection and/or restoration purposes. Most commonly used and reported natural modulators are triterpene and stilbenes (Lupeol, Fagarsterol, Resveratrol, Malibatol A, Geraniin, Compound A (Cpd A), Paeoniflorin-6'-O-benzene sulfonate, Aloe-emoidin) [15,53,70,71,75,100,111], flavonoids (Quercetin, Curcumin, Naringenin, Apigenin, Chrysin, Procyanidin, Epigallocatechin gallate, Apocynin, Paeonol) [20,27, 28,45,47,49,60,82,86,98,99,106]; terpenes (Forskolin or Coleonol, Triptolide, Terpinen-4-ol) [18,41,57,66] and alkaloid – Berberine [23,77]. To date, other synthetic immune modulators have been evaluated e.g. mono unsaturated fatty acid (*Cis*-palmitoleate) [14], Fluoroquinolone (Besifloxacin) [58], dietary supplement (Niacin) [107], Catecholamine (Dobutamine) [52], Cilostazol and β-ionone [75] in LPS-stimulated mouse alveolar macrophages. Both natural and synthetic immune modulators are evaluated in pre-clinical approaches.

Conclusions

Lipopolysaccharide-based inflammation produces neurotoxic (M1) and neuroprotective (M2) microglia. The M1 phenotype promotes cellular stress in the early stages of the disease, while M2 microglia repair or block the brain damage progression. Some natural and synthetic modulators that could favour the M1/M2 conversion have been reported, but robust evidence regarding cell signalling must be completed. Furthermore, the morphological changes and cytokines releasing profiles help to describe the microglial dynamics, but the proper identification of the M1 : M2 ratio would represent the real challenge and position of disease, thus would better promote health of the individual.

Disclosure

The authors report no conflict of interest.

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