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Natural Small-molecule Compounds Prevent Immune Imbalance *via* Regulating Microglia in Alzheimer's Disease*

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Abstract Alzheimer's disease (AD) is the most common neurodegenerative disorders of the central nervous system (CNS). Its pathogenesis is complex and needs to be further studied. Recent findings have shown that inflammation, which is characterized by the excessive activation of microglia to produce inflammatory factors, is closely related to the pathogenesis of AD. Improper regulation of microglial receptors and their downstream pathways impair immune homeostasis in AD patients and AD experimental animals. Natural small-molecule compounds reverse the immune imbalance and retard the progresses of AD *via* inhibting the microglial pro-inflammatory receptors, activating microglial anti-inflammatory receptors and regulating the receptors involved in Aβ clearance. In this paper, we reviewed the mechanisms of chronic inflammation caused by microglia in AD, and summarized the beneficial effects of natural small-molecule compounds in AD immune homeostasis by regulating microglial receptors and their downstream pathways.

Key words Alzheimer's disease, β-amyloid, inflammation, natural small-molecule compounds, homeostasis, microglia
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Alzheimer's disease (AD), one of the main types of senile dementia, is the most common degenerative disease of the central nervous system[1]. The factors associated with the increased risk of AD include genetic factors and non-genetic factors, among which age is the highest risk factor and the most common etiological factor[2]. The characteristic pathological changes of AD are extensive cerebral cortex atrophy, senile plaques, neuronal fibrillary tangles, synaptic signal changes and synaptic loss, extensive apoptosis, and degeneration of neurons[3]. The hypotheses of AD include the β-amyloid (Aβ) cascade hypothesis, the inflammation theory, the cholinergic system theory, the tau process hypothesis, the oxidative stress reaction theory, the free radical theory, the neuronal apoptosis theory, and the impaired synaptic plasticity theory[4]. The inflammation theory, which is characterized by the excessive activation of microglia and the production of inflammatory cytokines and free radicals, is closely related to the pathogenesis of AD[5]. The inflammatory reaction in AD patients may be a kind of chronic inflammation. However, acute reactive protein and inflammatory cytokines storm are found in the brain of AD patients. It leads to non-specific inflammatory cell infiltration (that is, the exudation of inflammation phases), chronic inflammation and damage to the central nervous system (CNS)[6-7]. Natural small-molecule compounds have beneficial effects on inhibition of the inflammatory cytokines storm and retard the formation and development of AD[8-9]. Recently,

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several experimental and clinical studies have validated the therapeutic effect of natural small-molecule compounds, highlighting their protective role and underlying mechanism in AD[10-14]. In this paper, we reviewed the progress of the beneficial effects of natural small-molecule compounds in the exudation of inflammation phases *via* maintaining immune homeostasis in AD.

1 The inflammatory response is a key factor in the pathogenesis of Alzheimer's disease

Neural inflammatory response plays an important role in neuronal damage and the subsequent progress of AD. The cells involved in neuroinflammation are microglia, astrocytes and neurons, and the inflammatory process is primarily mediated by activated microglia[15]. When CNS is damaged, microglia in a resting state are transformed into an activated state[16]. The activated microglia release many inflammatory cytokines that participate in the pathogenesis of AD through a variety of mechanisms.

Firstly, the inflammatory response is involved in cascade of Aβ. Aβ plays an important role in the pathogenesis of AD. Aβ can be divided into soluble Aβ, fibrous Aβ, and can form diffuse plaque or fibrous plaque[17]. There are a large number of activated microglia and inflammatory cytokines around the plaque, indicating that inflammation is involved in the occurrence and development of AD pathology. Aβ can directly activate microglia[18]. There are two different types of microglia, M1 and M2[19]. At early stages of AD, the activation of microglia by Aβ results in its transform to M2. M2 ingest soluble Aβ in a non-specific manner, through an unsaturated macro-pinocytosis pattern, and then transport it to the lysosome for rapid degradation. Phagocytosis of the Aβ by M2 through the pattern recognition receptor (PRR) is followed by the fusion of phagosomes and lysosomes, and degradation of the fibrous Aβ by lysosomal enzymes. M2 secrete anti-inflammatory cytokines that play an important role in maintaining immune homeostasis[20]. As the disease progresses, the accumulation of extracellular Aβ increases, consequently destroying immune homeostasis and inhibiting the activation of M2. This results in an increase in the proportion of M1 and releasing of pro-inflammatory cytokines[20]. Pro-inflammatory cytokines increase the expression of Aβ and further promote the activation of M1, resulting in a vicious cycled signaling cascade.

Secondly, the inflammatory response is also involved in the phosphorylation of tau. Tau plays an important role in synaptic synthesis and nuclear-cytoplasmic transport of neurons[21]. Under normal physiological conditions, the phosphorylation of tau is maintained at a relatively low level, and the phosphorylation and dephosphorylation is in an equilibrium state[22]. Microglia and astrocytes are actived and inflammatory cytokines are released in the brain of AD patients. They affect the selective splicing and chemical modification of tau, resulting in the hyperphosphorylation of tau in neurons[22]. Hyperphosphorylated tau do not associate with microtubules, but instead interact with each other to form oligomers. Then they combine to form a double-stranded helix that results in neurofibrillary tangles (NFTs), and consequently affects the occurrence and progression of AD[22].

Thirdly, the inflammatory response is involved in neuronal loss and decreased synaptic plasticity. Plasticity is an important part of cognition as well as the ability of the brain to respond to injury[23]. The loss of neurons and the decrease of synaptic plasticity are mainly caused by Aβ oligomers, which are highly cytotoxic forms of Aβ[24]. Microglia are involved in abnormal synaptic remodeling and synaptic loss caused by oligomers of Aβ, activated microglia release pro-inflammatory cytokines which have a direct excitotoxic effect on synapses[25]. The pro-inflammatory cytokines that are produced by microglia decrease synaptic plasticity and accelerate synapse degeneration and atrophy[26].

Finally, the inflammatory response is also thought to play a key role in oxidative stress. Under normal circumstances, the production and clearance of free radicals in the body is balanced. Upon exposure to harmful stimulation, reactive oxygen species (ROS) are produced in excess[27]. As the degree of oxidation exceeds the scavenging rate of oxide, the accumulation of free radicals results in varying degrees of body damage, termed oxidative stress. Inflammation is involved in oxidative stress in AD[27]. High-level expression of Aβ can transform the acute inflammatory reaction, originally aimed to protect the body, into chronic inflammatory injury *via*

the continuous activation of the inflammatory repair systems[6]. Long-term activated microglia release pro-inflammatory cytokines, including interleukin-1(IL-1), interleukin-6(IL-6) and tumor necrosis factor-α (TNF-α)[7]. ROS *in vivo* can activate redox sensitive transcription factors such as nuclear factor kappa-B (NF-κB), heat shock transcription factor 1(HSF1), and p53. It up-regulates the production of pro-inflammatory cytokines and leads to chronic inflammation[6-7]. Chronic inflammation can lead to an increase in ROS production, further enhancing oxidative stress and damage on the CNS.

2 Improper regulation of microglial receptors and their downstream pathways impair immune homeostasis in AD

Microglia can express many kinds of receptors. If these receptors and their downstream pathways are improperly regulated, immune homeostasis will be impaired and can lead to the development of AD. Studies have shown that a series of PRR expressed in microglia can be activated by Aβ[28].

Microglia receptors are classified into three types based on the effect after their activation: the pro-inflammatory receptors, the anti-inflammatory receptors and the receptors involved in Aβ phagocytosis. The pro-inflammatory receptors in microglia release pro-inflammatory cytokines after binding to Aβ, causing a subsequent pro-inflammatory signal cascade reaction and neurotoxicity[29-31]. The anti-inflammatory receptors in microglia can increase the expression of anti-inflammatory cytokines or decrease the expression of pro-inflammatory cytokines, alleviating the process of inflammation[32-33]. The receptors involved in Aβ phagocytosis are important for Aβ clearance[34]. Improper regulation of these receptors can cause inflammation and impair immune homeostasis in AD.

2.1 Increase release of inflammatory cytokines *via* activation of microglial pro−inflammatory receptors and their downstream pathways in AD

Aβ will activate the pro-inflammatory receptor of the microglia and induce the release of downstream inflammatory cytokines[29]. In AD patients, the release of these cytokines can lead to non-specific inflammatory cell infiltration, chronic inflammation and CNS damage, and other neurotoxic effects[30]. Additionally, the activation of the pro-inflammatory receptor will result in the secretion of even more pro-inflammatory cytokines *via* the up-regulation of microglia activity[30]. In particular, the pro-inflammatory receptors that have been associated with the progression of AD include the following receptors: receptors for advanced glycation end products (RAGE), Toll-like receptors (TLRs), NOD-like receptors (NLRs)[31,35-36] (Figure 1).

RAGE is a type of PRR[37]. Yan *et al.*[31] first reported that Aβ is the ligand of RAGE. In the brain of AD patients, the binding of Aβ to RAGE promote the migration of microglia to amyloid plaques and activate NF-κB (RelA/p50)[38]. NF-κB can up-regulate the expression of β -site APP cleaving enzyme 1 (BACE1) and increase the expression of Aβ, which subsequently leads to a series of cascade reactions. RAGE is one of the target genes transactivated by NF-κB. Additionally, the activation of microglia, induced by the interaction between RAGE and Aβ, is related to the p38 mitogen-activated protein kinase (MAPK) signaling[39]. Activation of RAGE will also further activate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. It increases the production of ROS, amplifies the production of Aβ, thus inducing inflammation[40]. This vicious cycle of inflammation promotes the transformation of acute inflammation into a chronic inflammatory process, and ultimately advances the disease progression of AD[39-40].

TLRs, are expressed in neural precursor cells (NPCs), neurons and glial cells. TLRs participate in immune function, the maturation of microglia, and the differentiation and development of neurons[41]. It can recognize pathogen-associated molecular patterns (PAMPs) such as Aβ, heat shock protein (HSP), and endotoxin[34]. Studies have shown that TLRs expressed in microglia are involved in the pathological process of AD. Coordination between myeloid differentiation protein-2 (MD-2) and CD14 facilitates the activation of TLRs[42]. In the brain of AD patients and AD animal models, a high expression of CD14, TLR2, TLR4 has been detected in microglia that are associated with Aβ[43]. The activation of TLRs by Aβ has effects on the myeloid differentiation primary response gene 88 (MyD88) pathway and the TIR-domain-containing adapter-inducing interferon-β (TRIF) pathway. Both pathways lead to the release of pro-inflammatory cytokines[43]. TLR2 relies on the MyD88 pathway[44]. It can either directly activate NF-κB (RelA/p50)[45], or indirectly, *via* activation of

p38MAPK through TNF receptor associated factor 6 (TRAF6), regulate NF- κB, and produces a downstream inflammatory response[46]. In addition to being associated with MyD88 signaling, TLR4 also recruits TRAF3/6 through TRIF to activate an inhibitor of nuclear factor kappa-B kinases (IKKs)[47]. The inhibition of these kinases increases expression of the downstream interferon regulatory factor (IRF3) and regulates the inflammatory response. TLR4 can also up-regulate the inflammatory response, either by activating TRIF to recruit TRAF6, or directly *via* the activation of NF- κB by translocation associated membrane protein (TRAM) [48]. These inflammatory cytokines can lead to negative effects on the micro-environment in the brain, and finally result in neuronal injury even death .

NLRs is classified as a PRR. In microglia, nod-like receptor protein 3 (NLRP3) is thought to play an important role[36]. NLRP3 is a kind of inflammasome that is composed of a variety of relevant proteins contained. These proteins include a sensor protein NLR, an adaptin apoptosis-associated speck-like protein containing a CARD (ASC), and a cysteine-requiring aspartate protease-1 (caspase-1) [49]. ASC provides a link between the upstream NLRP3 and the downstream caspase-1. In AD, microglia phagocytose extracellular oligomeric Aβ, resulting in lysosomal damage and release of cathepsin B[49]. Cathepsin B activates NLRP3 signaling. Downstream activation of NLRP3 signaling consists of two parts: a. the stimulation of NLRP3 by Cathepsin B, followed by the transcription of precursor proteins, such as pro-IL-1β and NLRP3 through the NF-κB pathway[50], and b. the activation of caspase-1 *via* the assembly of the NLRP3 inflammasome. Caspase 1 cleaves the precursors of IL-1β and IL-18 and produces mature inflammatory cytokines IL-1β and IL-18[49]. Once processed, modified, and mature, the downstream inflammatory cytokines are secreted. This results in an inflammatory response in brain.

2.2 Decrease expression of anti−inflammatory receptors in microglia aggravate the imbalance of inflammatory cytokines in AD

There are also receptors activaiton that release anti-inflammatory cytokines, or decrease the expression of pro-inflammatory cytokines to inhibit inflammation, such as triggering receptor expressed on myeloid cells 2 (TREM2) and α7-nicotinic acetylcholine receptor (α7nAChR)[32-33] (Figure 1).

TREM2 is a member of immunoglobulin-like receptor (KIP)[32]. TREM2 is specifically expressed in microglia, and its expression in microglia is more than 300 times higher than in neurons or astrocytes[51]. The over-expression of TREM2 can inhibite the release of inflammatory cytokines, and subsequently cause a increase in the survival rate of microglia[52]. In AD, the abnormal deposition of Aβ activates the TREM2[53]. TREM2 then phosphorylated the DNAX-activating protein of 12 ku (DAP12)[54]. Subsequently, Src kinase phosphorylates the immunoreceptor tyrosine-based activation motif (ITAM) tyrosine residues of DAP12, which forms the docking site of spleen tyrosine kinase (Syk), and then phosphorylates Syk[54]. Phosphorylated Syk further phosphorylates the scaffold molecules L-type amino acid transporter (Lat) or recombinant human non-T-cell activation linker (NTAL), to recruit proximal signal molecules. TREM2 regulates signal transducer and activator of transcription (STAT) and up-regulates the expression of anti-inflammatory cytokines *via* TREM2/DAP12 signaling[54]. *Via* the activation of the TREM2/DAP12 signaling, TREM2 can also regulate the phagocytic function of microglia by activating phosphatidylinositol 3 kinases (PI3K) and extracellular regulated protein kinase (ERK1/2), respectively[54]. TREM2 is also thought to inhibit the release of pro-inflammatory cytokines by suppressing TLR4. The increase of TREM2 expression reduces the expression of CARD9, and consequently reduces the binding of CARD9 to p38MAPK, thereby down-regulating the inflammatory response[55].

nAChR is thought to be involved in inflammation[33]. Studies have shown that α7 subtype is that mainly expressed in the hippocampus[56]. α7nAChR belongs to the ligand-gated ion channel (LGIC) family, and its main function is to recognize the signals transmitted *via* cholinergic neurotransmitters[57]. The affinity between α7nAchR and Aβ is very strong in microglia[58]. α7nAChR agonist can promote the influx of Ca^{2+} and activate PI3K-ERK1/2-CREB signaling. It significantly alleviates the deposition of Aβ, which in turn reduces the expression of inflammatory cytokines, thus protecting learning and memory function[59]. The contain of Aβ40 and Aβ42 in hippocampus of α 7nAchR gene knockout APPS transgenic mice significantly increased[60]. When α7nAchR is pre-

activated in the microglia which is co-cultured with neurons with transwell, the degree of neuronal injury is reduced even further[61]. In addition, the activation of α7nAChRs inhibits the transformation of M1. It can promote the M2 and contribute to the modulation of nerve neuroinflammation during several CNS diseases such like AD[62].

2.3 Improper regulation of the Aβ clearance receptor in microglia promote the progress of AD

The receptor involved in Aβ clearance are scavenger receptors (SRs)[34]. Inappropriate regulation of the Aβ clearance receptors in microglia will aggravate the pathological process of AD (Figure 1).

SRs are a type of cell surface glycoprotein that lead to clearance and internalization of damaged cells, tissues, proteins and cell fragments, *etc*. They can bind to Aβ[63]. Subtypes of SRs include SR-A, SR-B, SCARC, CD68, lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and SCARF. The two main types of SRs in the CNS are SR-A and CD36. SR-A is mainly expressed in microglia and astrocytes, while CD36 is mainly expressed in microglia and endothelial cells[34].

Scara1 is a member of SR-A. SR-A plays a role in the immune surveillance of endogenous harmful substances[63]. Early studies of Scara1 have shown that it can mediate the uptake of Aβ in microglia, and as a result, facilitate the decomposition and clearance of Aβ[64]. Additionally, the expression level of Scara1 in the brain of AD mice decreases with age. It serves as further evidence that Scara1 may affect the deposition of Aβ[65]. According to the results of Frenkel *et al*.[66], the expression of Scara1 in microglia *in vitro* promotes the phagocytosis of soluble Aβ. Additionally, in PS1-APP-Scara1-deficient mice, Scara1 deficiency is thought to accelerates the accumulation of Aβ, leading to increased mortality[66]. In terms of the downstream effects of Scara1 in microglia, there are only a few studies, but p38MAPK signaling is a pathway that is thought to be involved[67].

CD36 is a member of SR-B. In the CNS, CD36 is mainly expressed on the surface of microglia. Earlier *in vitro* studies have shown that CD36 on microglia can bind to fibrous or soluble Aβ, promote the release of inflammatory cytokines, and mediate the oxidative stress response[68]. The binding of Aβ to CD36, activates p38MAPK/NF- κB signaling[69]. In addition, Wang *et al*.[70] demonstrated that the nuclear factor erythroid 2-related factor 2 (NRF2), a major component regulating antioxidant response, increases CD36 expression. The increase in CD36 expression is thought to improve the weak antioxidant response triggered by hypoxia, diminish Aβ deposition, and improve spatial memory defects. As mentioned earlier, activated NLRP3 promotes the cascade reaction and inflammatory response in AD. It has been found that CD36 plays an important role in regulating the activation of the inflammasome by Aβ[70]. Aβ enters the microglia via the action of CD36, accumulates in the lysosome, and activates the inflammasome[71]. In addition, Aβ binds to CD36 and forms receptor complexes with other receptors, further driving downstream signal cascade reactions that then lead to the production of ROS[72]. Overall, these results suggest that CD36 plays an important role in the chronic inflammation that is a key factor in the pathological process of AD.

3 Natural small-molecule compounds regulate the pathological process of Alzheimer's disease by maintaining immune homeostasis

Natural small-molecule compounds can regulate the three types of receptors described above, thus maintaining immune homeostasis and relieving the pathological process of AD.

3.1 Natural small-molecule compounds inhibit the activation of microglial pro-inflammatory receptors and their downstream pathways in AD

Natural small molecule compounds have been shown to play a role in the regulation of RAGE. The components of natural small-molecule compounds that have been shown to target RAGE and effectively alleviate chronic inflammation of AD include, iridoid glycosides (geniposide), saponins (salidroside, pseudoginsenoside-F11), alkaloids (matrine, ligustrazine), flavonoids (apigenin, soybean isoflavones), polyphenols (resveratrol, curcumin, apple polyphenol phloretin) and terpenoids (ursolic acid)[10-11,73-81](Figure 1).

Geniposide (compound 1 in Table 1) inhibits the binding of Aβ to RAGE in the brain of AD transgenic mice, thus inhibiting MAPK-NF- κB, lowering the level of IL-1β, TNF- α, ICAM-1, VCAM-1, and

chemokine MCP-1[10]. Salidroside (compound 2 in Table 1) has been shown to inhibit the expression of RAGE in AD model rats, affect NF-κB signaling and inhibit the production of iNOS in the hippocampus[73]. Matrine (compound 3 in Table 1) can prevent the binding of Aβ to RAGE in the brain of APP/PS1 mice and reduce the expression of TNF-α, IL-1β and Aβ plaque deposition[11]. Studies of the the neuroprotective effect of ligustrazine (compound 4 in Table 1) on Aβ1-40 induced rats, have shown that it can effectively prevent inflammatory injury by inhibiting RAGE-p38-NF-κB signaling, decrease the expression of TNF-α, IL-1β and IL-6, and lower the amount of Aβ plaque deposition[75]. Apigenin (compound 5 in Table 1) attenuates the impairment in learning and memory induced by Aβ1-42. It can reduce the expression of GFAP, Iba-1, HMGB1, RAGE and p65 NF-κB, and has a protective effect on nerves *via* the regulation of the inflammatory response in AD[76]. Soybean isoflavones (compound 6 in Table 1) can reduce the contents of RAGE and IL-6 in the hippocampus. Additionally, they can attenuate the inflammatory effect and the Aβ toxic injury, as well as resist neuronal apoptosis[77]. Resveratrol (compound 7 in Table 1) reduces the expression of RAGE in the hippocampus, changes the expression of NF-κB, and protects the integrity of blood-brain barrier (BBB) in AD model rats[78]. Through a mechanism that is related to the inhibition of the expression and extracellular release of HMGB1, curcumin (compound 8 in Table 1) can reduce the inflammatory response of BV2 induced by Aβ25-35 , the expression of NF-κB, RAGE, ROS, lipid peroxidation (LPO) and Nrf 2/HO^{-1}[79]. Apple polyphenol phloretin (compound 9 in Table 1) can produce an anti-inflammatory effect through RAGE/p38MAPK/NF-κB signaling, and consequently inhibit the formation of AGEs, which is stimulated by methyl acetaldehyde (MGO) [80]. The elevated levels of NLRP3 were reduced by Pseudoginsenoside-F11 (PF11) (compound 10 in Table 1) through reducing the accumulation of AGEs, the expression of the RAGE and the level of Nrf2 and glutathione S-transferase (GST) in hippocampus. Moreover, PF11 significantly decreased H_2O_2 and malondialdehyde (MDA) levels, improved superoxide dismutase (SOD) activity and increased glutathione (GSH) level[74]. Ursolic acid (UA) (compound 11 in Table 1) significantly improved behavioral performance of D-gal-treated mice in step-through test and Morris water maze task. It decreased the expression of iNOS, COX-2, ROS, and decreased IL-1β, IL-6, TNF-α levels. Furthermore, the results showed that UA reduced the number of activated microglia, downregulated AGEs and induced the expression of RAGE also NF-κB p65[81].

Natural small-molecule compounds are also involved in the regulation of TLRs. As a result, natural small-molecule compounds can be used as targets for TLRs to alleviate the chronic inflammation of AD. Some examples of effective natural small-molecule compounds include terpenoids (celastrol), flavonoids (soybean isoflavones, genistein, dihydromyricetin), alkaloids (evodiamine), coumarins (osthol), saponins (ginsenoside Rg1, xanthoceraside, astragaloside IV), polyphenols (epigallocatechin-3-gallate) and iridoid glucoside (catalpol) [12-13,82-90] (Figure 1).

By preventing Aβ from binding to TLR4/MD2 complex, celastrol (compound 12 in Table 1) can decrease the level of pro-inflammatory cytokines. It can also reduce the level of tau hyperphosphorylation in cells induced by Aβ1-42[12]. Soybean isoflavones (compound 13 in Table 1) have been shown to reduce the expression of pro-inflammatory cytokines. It can lower the expression of TLR4 as well as NF-κB in the hippocampus, thus attenuating the impairment of spatial learning and memory induced by Aβ1-42 in Wistar rats[13]. Genistein (compound 14 in Table 1) can reduce the level of TLR4, TNF-α, COX2, iNOS, glial fibrillary acidic protein (GFAP), and increase the level of Nrf2 by inhibiting TLR4/NF-κB signaling[82]. It is proved that Dihydromyricetin (DHM) (compound 15 in Table 1) can reduce the inflammatory response of BV-2 induced by lipopolysaccharide (LPS), inhibit the expression of IL-6, IL-1β, TNF-α, iNOS and COX-2 through TLR-4/NF-κB signaling[83]. Evodiamine (compound 16 in Table 1) can protect the nervous system by inhibiting the TLR4/NF-κB signaling and reducing the neuroinflammatory injury in AD rats[84]. Osthol (compound 17 in Table 1) downregulates the expression of TLR4, TRAF6, as well as NF-κB[85]. By blocking the signal transduction pathway of TLR4, Ginsenoside Rg1 (compound 18 in Table 1) can reduce the production of TLR4, NF-κB, TRAF6, TNF-α, IFN-β and iNOS of NG108-15 induced by Aβ25-35, thus protecting cells from neuroinflammation[86]. Xanthoceraside (compound 19 in Table 1) has been

shown to down-regulate TLR2 in microglia and inhibit the expression of IKKs, which results in the degradation of IκB protein, and NF-κB p50/p65[87]. It showed that Astragaloside IV (AST-IV) (compound 20 in Table 1) can partially protect LPS-induced microglia from death and downregulate the release of IL-1β, IL-6, TNF-α, NO, and decrease the expression of TLR4, MyD88 and NF-κB in microglia. Moreover, AST-IV can increase the production of anti-inflammatory cytokines IL-10 and arginase 1, an M2 marker of microglia[88]. Epigallocatechin 3 Gallate (EC3G) (compound 21 in Table 1) inhibited the expressions of Iba-1, IL-1β and IL-18 induced by LPS+Aβ in BV2. Furthermore, the expression levels of TLR4, p-IKK/IKK, and p-NF- κB/NF- κB also decreased after EGCG treatment. EC3G reduced the expression of caspase-1, NLRP3 and cleaved caspase-1, reducing microglial inflammation and neurotoxicity[89]. Catalpol (compound 22 in Table 1) inhibited the expression of TNF-α, IL-1β also ROS in LPS-induced BV2. In addition, catalpol suppressed the NF- κB signaling by interfering with the phosphorylation and degradation of iκB- α and blocking the NF-κB p65. Moreover, catalpol inhibited the expression of TLR4 and MyD88. These indicate that catalpol can have potential benefits by inhibiting the occurrence of inflammation[90].

Natural small-molecule compounds are also involved in the regulation of NLRs, some examples include lignans (schisandrin), polyphenols (resveratrol), terpenoids (nootkatone, lupeol), lavonoids (baicalina), flavonoids (corylin, dihydromyricetin), phenolic acid (rosmarinic acid) and saponins (esculentoside A)[14,91-98] (Figure 1).

After treatment with schisandrin (SCH) (compound 23 in Table 1), the expression of TNF-α, IL-1β and IL-6 was shown to decrease, thus alleviating the inflammatory response *via* the inhibition of the TLR4/NF- κB/NLRP3 signaling[14]. Additionally, SCH decreased significantly restore the activities of SOD, GST, COX-2, total antioxidant capacity (T-AOC), iNOS, GSH, malondialdehyde (MDA) and NO. It has been found that nootkatone (NKT) (compound 24 in Table 1) decrease the high expression of IL-1β, IL-6, TNF-α, NLRP3 and NF-κB p65, especially in hippocampus[92]. Resveratrol (compound 25 in Table 1) can inhibit NLRP3 signaling and reduce the inflammatory response of BV-2 stimulated by Aβ. It has also been shown to suppress the overexpression of cleaved caspase-1, IL-1β, thus abrogating Aβ -stimulated degradation of IκBα and phosphorylation of NF- κB[92]. Lupeol (compound 26 in Table 1) can down-regulate the expression of TNF, iNOS and NLRP3, and inhibit the neuroinflammatory response induced by LPS in Wistar rats. In addition, lupeol was shown to up-regulate the expression of the neurotrophins, glial cell line-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) [93]. Baicalin (BAI) (compound 27 in Table 1) has been shown to elicit strong anti-inflammatory and neuroprotective effects. It can improve memory and cognitive impairment in APP/PS1 mice by inhibiting the activation of NLRP3 signaling, as well as the neuroinflammatory response in microglia[94]. Results show that corylin (compound 28 in Table 1) inhibit the production of NO and pro-inflammatory cytokines by LPS-activated BV2. Corylin can inhibit the expression of iNOS and COX-2, decrease the expression of NLRP3 and ASC, and inhibit the activation of caspase-1 and IL-1β[95]. DHM (compound 29 in Table 1) treatment could significantly improve the memory and cognitive impairment of APP/PS1 mice and reduce the number of microglia activated in hippocampus and cortex. Furthermore, DHM can promote the clearance of Aβ by increasing the level of neprilysin (NEP), decreased the expression of NLRP3 and transform microglia into M2[96]. Rosmarinic acid (compound 30 in Table 1) reduced the expression of CD11b, a marker of microglia and macrophages. It inhibited the levels of TNF- α, IL-6, IL-1β and COX-2, and suppressed the activation of the NF- κB/NLRP3 signaling[97]. Esculentoside A (EsA) (compound 31 in Table 1) pretreatment significantly decreased the production of NO and Prostaglandin E2 (PGE2), reduce the expression of iNOS, COX-2, IL-1β, IL-6, IL-12 and TNF-α in both BV2 and primary microglia. Moreover, EsA inhibited the translocation of NF- κB p65 by blocking IκB- α phosphorylation in LPS-treated BV2. EsA also decreased phosphorylation level of MAPKs and inhibited the activation of caspase-1 mediated by NLRP3 in BV2[98].

3.2 Natural small−molecule compounds activate microglial anti−inflammatory receptors and its downstream pathways in AD

The active components of natural small-molecule compounds targeting TREM2 to reduce chronic inflammation of AD have also been reported, and the effect of polyphenols (resveratrol, curcumin) and alkaloids (paclitaxel) on TREM2 have been studied at length[99-101] (Figure 1).

Resveratrol (compound 32 in Table 1) has been shown to promote phagocytosis in BV2 as well as regulate the expression and transport of TREM2. Additionally, it can reduce the mRNA level of IL-1β in BV-2 induced by Aβ[99]. Curcumin (compound 33 in Table 1) significantly alleviated LPS-induced inflammation by regulating microglial (M1/M2) polarization, and maintained the balance of TREM2 and TLR4. It has also been shown to regulate the downstream NF-κB signaling[100]. Findings by Inoue T et al. have shown that paclitaxel (compound 34 in Table 1) can inhibit the production of Aβ by inhibiting ERK1-APP. It can regulate the expression of TREM2, reduce the level of glutamate and oxidative injury[101]. The natural small-molecule compounds that are related to TREM2 are rarely reported. TREM2 not only promotes phagocytosis of Aβ by microglia, but also upregulates the expression of anti-inflammatory cytokines, and inhibits the downstream pathway of TLR4. TREM2 has been a major topic of research in recent years, and will be an ideal drug target in the future.

Natural small-molecule compounds have been shown to play a role in the regulation of α7nAChR receptor. There are few reports on the natural small-molecule compounds targeting α7nAChR to alleviate the chronic inflammatory reaction of AD, including saponins (tenuigenin), alkaloids (galanthamine) and flavonoids (rhamnetin, scutellarin)[102-105] (Figure 1).

Recent findings have shown that when treated with tenuigenin (TEN) (compound 35 in Table 1), the spatial learning and memory ability of Wistar rats induced by D-galactose was significantly improved. Moreover, TEN has been shown to significantly increase the expression of α7nAChR in a dose dependent manner in microglia[102]. Galanthamine (compound 36 in Table 1) can inhibit cholinesterase activity, regulate the activity of α7nAChR in microglia, inhibit potassium channels, and activate dopamine receptors. It has been used in clinical treatment of AD because its sensitizes microglial α 7nAChR to choline and induces Ca^{2+} influx into microglia[103]. Rhamnetin (compound 37 in Table 1) can target α7nAChR, and reduce downstream inflammation in microglia[104]. Scutellarin (Scu) (compound 38 in Table 1) can improve the learning and memory ability and increased the expression of α 7nAChR in rats with dementia. It is suggested that the mechanism may be related to its up-regulation of α 7nAChR expression[105].

3.3 Natural small−molecule compounds show its benefit effects through regulate the receptors involved in Aβ clearance in AD

Natural small-molecule compounds participate in the regulation of the receptors called SRs. It has been speculated that, up-regulating the expression of Scara1 on microglia membrane may be an effective target for AD therapy[106-107].

Ginsenoside Rg3 (compound 39 in Table 1) has been shown to enhance the ability of microglia to phagocytose Aβ, and up-regulate the expression of SR-A in a dose-and time-dependent manner[106]. In addition, both NEP and insulin-degrading enzyme (IDE) were highly expressed by Rg3[107]. UA (compound 40 in Table 1), has been shown to inhibit the binding of Aβ to CHO-CD36 in a dose-dependent manner, block binding of Aβ to microglia, and thus reduce the subsequent production of ROS. Through its ability to block Aβ-CD36 interactions, UA could be have therapeutic potential for the treatment of AD[108] (Figure 1). *M. parviflora* leaf hydroalcoholic extract (MpHE) (compound 41 in Table 1) contains the active phytochemicals, oleanolic acid and scopoletin. MpHE can increase the expression of TREM2 through the PPAR-γ/CD36-dependent mechanism, effectively reducing the accumulation of insoluble Aβ in microglia and the hippocampus, and as a result, lowering the expression of M1 inflammatory marker[109].

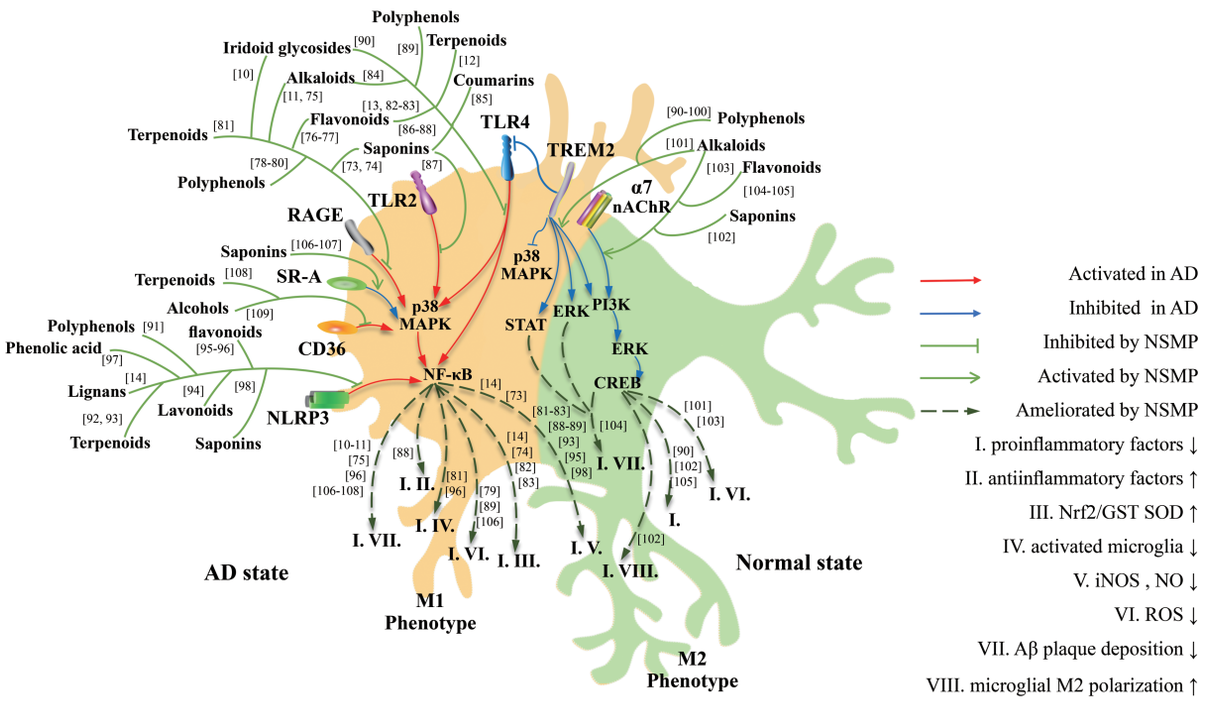

Fig. 1　The target receptors and underlying signal pathways of natural small−molecule compounds in microglia

The pro-inflammatory receptors and theirs downstream signal pathways are over activated in AD (red line). While anti-inflammatory receptors and theirs downstream signal pathways are inhibited (blue line). Natural small-molecule compounds either through inhibiting NLRP3/NF-κB signaling, CD36/NF-κB/p38 MAPK signaling, RAGE/NF-κB/p38 MAPK signaling, TLR2/NF-κB/p38 MAPK signaling, TLR4/NF-κB/p38 MAPK signaling or promoting the activation of Scara1(SR-A)/NF- κB/p38 MAPK signaling, TREM2/STAT/PI3K/ERK signaling and α7nAChR/PI3K/ERK/CREB signaling alleviating the downstream inflammatory response and the related pathological symptoms in AD.

Table 1　Natural small−molecule compounds regulate the pathological process of Alzheimer's disease by maintaining immune homeostasis

Number	Effective natural small-molecule compounds	Targeted receptor	Targeted signaling pathway	Results
1	Geniposide [10]	RAGE	RAGE/ MAPK-NF-κB	proinflammatory factors (IL-1β, TNF-α) ↓ adhesion factors (ICAM-1, VCAM-1) chemokine MCP-1 ↓
2	Salidroside [73]	RAGE	RAGE/NF-κB	iNOS ↓ proinflammatory factors (IL-1β, TNF-α) ↓
3	Matrine [11]	RAGE	RAGE	TNF- α, IL-1β ↓ Aβ plaque deposition ↓
4	Ligustrazine [75]	RAGE	RAGE/ p38 MAPK-NF-κB	TNF-α, IL-1β and IL-6 ↓ Aβ plaque deposition ↓
5	Apigenin [76]	RAGE	RAGE/p65 NF-κB	GFAP, Iba-1, HMGB1 ↓ RAGE and p65 NF-κB ↓
6	Soybean isoflavones [77]	RAGE	RAGE	RAGE and IL-6 ↓
7	Resveratrol [78]	RAGE	RAGE/NF-κB	RAGE and NF-κB ↓

Continued to Table 1

Number	Effective natural small-molecule compounds	Targeted receptor	Targeted signaling pathway	Results
8	Curcumin [79]	RAGE	RAGE/NF-κB	HMGB1, NF-κB, RAGE ↓ ROS ↓
9	Apple polyphenol phloretin [80]	RAGE	RAGE/p38 MAPK/NF-κB	AGEs ↓ anti-inflammatory effect
10	Pseudoginsenoside-F11 [74]	RAGE/NLRP3	RAGE/NLRP3	NLRP3, AGEs ↓ Nrf2, GST, SOD, GSH ↑ H_2O_2, MDA ↓
11	Ursolic acid [81]	RAGE	RAGE/NF-κB	iNOS, COX-2, ROS ↓ IL-1β, IL-6, TNF-α ↓ activated microglia ↓ AGEs, RAGE, NF-κB p65 ↓
12	Celastrol [12]	TLR4	TLR4/NF-κB	TLR4/MD2 complex ↓ proinflammatory factors ↓ tau hyperphosphorylation ↓
13	Soybean isoflavones [13]	TLR4	TLR4/NF-κB	TLR4, NF-κB ↓
14	Genistein [82]	TLR4	TLR4/NF-κB	TLR4 ↓ TNFα, COX2 ↓ iNOS ↓ GFAP ↓ Nrf2 ↑
15	Dihydromyricetin [83]	TLR4	TLR4/NF-κB	IL-6, IL-1 β, TNF-α ↓ iNOS, COX-2 ↓
16	Evodiamine [84]	TLR4	TLR4/NF-κB	HMGB1, TLR4, NF-κB ↓
17	Osthol [85]	TLR4	TRAF6/TLR4/NF-κB	TRAF6, TRR4, NF-κB ↓
18	Ginsenoside Rg1 [86]	TLR4	TLR4	TLR3, TLR4, NF-κB, TRAF-6 ↓ TNF-α, IFN-β ↓ iNOS ↓
19	Xanthoceraside [87]	TLR2	TLR2/ MAPK-NF-κB	inflammatory factors ↓ IκB, p50, p65 ↓
20	Astragaloside IV [88]	TLR4	TLR4/MyD88/NF-κB	IL-1β, IL-6, TNF-α, nitric oxide ↓ TLR4, MyD88, NF-κB ↓ IL-10, arginase 1 ↑
21	Epigallocatechin-3-Gallate [89]	TLR4/NLRP3	TLR4/NF-κB NLRP3/caspase-1	Iba-1, IL-1β, IL-18 ↓ TLR4, p-IKK/IKK ↓ p-NF-κB/NF-κB ↓ caspase-1, NLRP3, caspase-11 ↓
22	Catalpol [90]	TLR4	TLR4/MyD88/NF-κB	iNOS, cyclooxygenase-2 ↓ TNF-α, IL-1β, ROS ↓ NF-κB, iκB-α, NF-κB p65 ↓ TLR4, MyD88 ↓
23	Schisandrin [14]	TLR4/NLRP3	TLR 4/NLRP 3/NF-κB	TNF-α, IL-1β, IL-6 ↓ SOD, GST ↑ COX-2, T-AOC ↓ iNOS ↓
24	Nootkatone [92]	TLR4/NLRP3	TLR 4/NLRP 3/NF-κB	IL-1β, IL-6, TNF-α ↓ NLRP3, NF-κB p65 ↓
25	Resveratrol [91]	NLRP3	NLRP 3/NF-κB	caspase-1, IL-1β IkBα, NF-κB ↓

Continued to Table 1

Number	Effective natural small-molecule compounds	Targeted receptor	Targeted signaling pathway	Results
26	Lupeol [93]	NLRP3	NLRP 3	TNF ↓ iNOS ↓ NLRP3 ↓ GDNF, NGF ↑
27	Baicalin [94]	NLRP3	NLRP 3	proinflammatory cytokines ↓
28	Corylin [95]	NLRP3	NLRP3/caspase-1	NO, proinflammatory cytokines ↓ iNOS, COX-2 ↓ NLRP3, ASC ↓ caspase-1, IL-1β ↓
29	Dihydromyricetin [96]	NLRP3	NLRP3	activated microglia ↓ NLRP3 ↓ agrinase-1, neprilysin, Aβ clearance ↑
30	Rosmarinic acid [97]	NLRP3	NLRP3/NF-κB	CD11b ↓ TNF-α, IL-6, IL-1β, COX-2 ↓ NF-κB, NLRP3 ↓
31	Esculentoside A [98]	NLRP3	NLRP3/NF-κB/caspase-1	NO, prostaglandin E2 , iNOS ↓ COX-2, IL-1β, IL-6, IL-12, TNF-α ↓ NF-κB p65, p-IκB-α, MAPKs ↓ caspase-1, NLRP3 ↓
32	Resveratrol [99]	TREM2	TREM2	TREM2 ↑ inflammatory factor ↓
33	Curcumin [100]	TREM2	TREM2-TLR4/NF-κB	microglial (M1/M2) polarization balance TREM2 and TLR4
34	Paclitaxel [101]	TREM2	TREM2/ERK1-APP	glutamate, oxidative injury ↓ active caspase ↓
35	Tenuigenin [102]	α7nAChR	α7nAChR	α7nAChR ↑ inflammatory factor ↓
36	Rhamnetin [104]	α7nAChR	α7nAChR	α7nAChR ↑ Ca(2+) influx ↑ Aβ plaque deposition ↓
37	Galanthamine [103]	α7nAChR	α7nAChR	free radical ↓ neuroinflammatory ↓
38	Scutellarin [105]	α7nAChR	α7nAChR	α7nAChR ↑ inflammatory factor ↓
39	Ginsenoside Rg3 [107]	SR-A	SR	Aβ plaque deposition ↓ inflammatory factor ↓
40	Ursolic acid [108]	CD36	CD36	Aβ plaque deposition ↓ ROS ↓
41	MpHE [109]	CD36	TREM2/PPAR-γ/CD36	Aβ plaque deposition ↓ inflammatory factor ↓

4 Summary and prospect

Microglia play an important role in the pathological process of inflammation. More and more natural small-molecule compounds have been shown to target microglial receptors and their downstream pathways, thus eliciting anti-inflammatory and neuroprotective effects. The studies, described in this review, have laid the foundation for the development of drugs for the treatment of AD. Screening innovative drugs for the treatment of AD from the effective natural small-molecule compounds is of great interest for future research. There has been a great deal of progress in the preclinical development

of Crocus sativus-Based Botanical Lead IIIM-141 for Alzheimer's Disease, and a sustained release(SR) capsule formulation has been developed[110]. Taisi capsule stilbene glycoside, the main active component of *Fallopia multiflora*, is one compound that is currently in Phase III clinical trials, Galantamine, a phenanthrene alkaloid extract extracted from *Lycoris radiate*, is currently being used in the clinical treatment of AD[103]. As a second generation cholinesterase inhibitor, galantamine can also effectively activate α7nAChR receptors to produce anti-inflammatory cytokines. Future research on the ability of small-molecule compounds to target multiple microglial Aβ receptors and play a synergistic role is of great interest. In addition, combination of small molecules with lipids, proteins and sRNAs as found in decoctosomes show a better therapeutic effect *in vivo*, it provides a new idea for the research and development of different medicine dosage form for the treatment of AD[111].

As compared to the prescription of traditional Chinese medicine, it is easier for natural small-molecule compounds to pass through the BBB and for subsequent clinical qualitative and quantitative analysis. Although great progress has been made in the study of microglial receptors targeted by natural small-molecule compounds in AD in recent years, natural small-molecule compounds and microglia may be a double-edged sword for AD, and there are still many questions that need to be resolved. If different natural small-molecule compounds are used together to target different receptors, just as in traditional Chinese medicine, will these compounds cross-react and what are the safety implications of interactions between these compounds? We still have a long way to go to solve these problems, but an in-depth study of them will certainly aid in understanding the role of natural small-molecule compounds in AD.

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天然小分子化合物调节小胶质细胞预防阿尔茨海默病免疫失衡*

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摘要　阿尔茨海默病（Alzheimer's disease，AD）是最常见的中枢神经系统退行性疾病，其发病机制复杂，至今仍未完全阐明. 最近的研究表明，小胶质细胞过度激活、炎症因子的过度产生与AD的发病密切相关. 小胶质细胞受体及下游通路的异常调节可导致AD患者及AD实验动物的免疫失衡. 天然小分子化合物通过激活小胶质细胞的抑炎受体，抑制促炎受体或调节Aβ清除受体，可逆转免疫失衡. 本文综述了小胶质细胞在AD慢性炎症中的作用机制，并总结天然小分子化合物通过调节小胶质细胞受体及其下游通路在AD免疫稳态中的有益作用.

关键词　阿尔茨海默病，β-淀粉样蛋白，炎症，天然小分子化合物，稳态，小胶质细胞
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