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The Toll-like Receptor 2 (TLR2)-related Immunopathological Responses in the Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis

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ABSTRACT

Toll-like receptors (TLRs) play principle roles in recognition of autologous components which have been pointed as the danger-associated molecular patterns (DAMP) and microbial components which are identified as pathogen associated molecular patterns (PAMP).The infiltration of various inflammatory cells such as dendritic cells, lymphocytes ($CD4^+$ T, $CD8^+$ T as well as B cells), monocytes and macrophages occur into the central nervous system (CNS) during multiple sclerosis (MS) and its animal model named experimental autoimmune encephalomyelitis (EAE). The infiltrated leukocytes and residential cells of the CNS express several TLRs (especially TLR2) and their expression are elevated in MS and EAE. TLR2 recognizes a large variety DAMP and PAMP molecules due to its ability to create heterodimers with TLR1, TLR6 and probably TLR10. A wide spectrum of DAMP molecules, including heat shock protein 60 (HSP60), HSP70, high mobility group box 1 (HMGB1), β-defensin 3, surfactant protein A and D, eosinophil-derived neurotoxin, gangliosides, serum amyloid A, hyaluronic acid and biglycan are identified by TLR2, whose their expression is increased in MS patients. TLR2 may contribute in the development of MS and EAE diseases through the reinforcement of Th1/Th17 cell-related responses, downregulation of regulatory T cells, induction of IL-17^+ γδ T cells, inhibition of oligodendrocyte maturation, induction of poly ADP-ribose polymerase-1 (PARP-1)-dependent pathway in microglia, macrophages and astrocytes and inhibition of type I interferons expression. The contribution of TLR2-related immunopathological responses in the MS and EAE pathogenesis and its possible targeting as promising therapeutic potentials are considered in this review.

Keywords: Experimental autoimmune encephalomyelitis; Multiple sclerosis, Pathogenesis; Toll like receptor 2

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INTRODUCTION

Multiple sclerosis (MS) is an autoimmune-mediated disease causes the neuronal demyelination and axonal injury in the central nervous system (CNS).[1] At present, about 2.5 million patients suffer from MS and women were affected by a greater risk than men (about 2/1), worldwide.[2,3] The clinical courses of MS are classified as relapsing-remitting (RRMS), primary progressive (PPMS), secondary progressive (SPMS) and progressive relapsing (PRMS).[4] The experimental model of MS disease has been identified as experimental autoimmune encephalomyelitis (EAE) which is inducible in a susceptible animal by active immunization with myelin-derived antigens, such as myelin oligodendrocyte protein (MOG), myelin basic protein (MPB) or proteolipid protein (PLP) emulsified in a suitable adjuvant.[5-7] EAE has been introduced as an appropriate model to explain the mechanisms of MS as well as therapeutic approaching to this disease.[8]

The autoimmune response against myelin constituents in CNS play an essential role in MS pathogenesis.[9]Pathologically, the perivascular infiltration of immune cells such as dendritic cells (DCs), lymphocytes ($CD4^+$and $CD8^+$T cells as well as B cells), monocytes and macrophages happen into the CNS during the initial stages of the disease. The infiltrated leukocytes contribute to the demyelination, astrocytosis and neuronal as well as axonal degeneration.[10]

In MS and EAE, the rupture of the blood-brain barrier (BBB) and migration of activated leukocytes occur into the CNS.[11] The DCs migrate across the inflamed BBB and trigger the differentiation of myelin specific naïve $CD4^+$ T lymphocytes into the various effector T cell subsets.[9,11] The demyelination process in MS and EAE is directed by the pathogenic forms of Th1- and Th17 cells,[9,12] whereas the regulatory T (Treg) cells can protect the human and animals against the autoimmune diseases.[9,12] The microglia as the CNS macrophages also participate in the immunopathological process of the MS and EAE diseases by producing proinflammatory cytokines and reactive molecules, for instance nitric oxide and reactive oxygen intermediates.[13] The $CD8^+$ cytotoxic T lymphocytes (CTLs) may directly cause axonal damage by releasing granzyme B and perforin.[3] Moreover, the B cells, as well as γ/δ T-cells, natural killer (NK) cells and innate lymphoid cells play a critical role in the immunopathological process of MS and EAE diseases.[3,14]

Toll-like receptors (TLRs) are the cell surface-linked proteins that belong to the pathogen recognition receptors (PRR) family in innate immunity. TLRs play a fundamental role in recognition of non-microbial endogenous ligands that are called danger-associated molecular patterns (DAMP) and conserved microbial constituents that are identified as pathogen associated molecular patterns (PAMP).[15,16] The intracellular-originated DAMP molecules, including heat shock protein 70 (HSP70), heat shock protein 90 (HSP90), high mobility group box 1 (HMGB1) and cellular RNA are released after tissue damage or cell death.[16] The PAMP molecules are originated from infectious agents such as lipoproteins, lipopeptides, peptidoglycans, lipopolysaccharides (LPS), CpG-motif-containing DNA (CpG-DNA), single- and double stranded ribonucleic acid.[16,17]

In total, 13 kinds of TLRs have been specified so far, in which TLR1 to TLR9 commonly exist in both humans and mice.[16]TLR10 has been exclusively designated in humans, whereas TLR11 to TLR13 only exist in mice. The microbial-derived PAMP such as LPS, lipopeptides, bacterial flagellum, viral-derived dsRNA, viral or bacterial-derived ssRNA and CpG-rich unmethylated DNA are recognized by TLR4, TLR2-TLR1 and TLR2-TLR6 heterodimers, TLR5, TLR3, TLR7/TLR8 and TLR9, respectively.[18] Activation of TLRs by PAMP or DAMP result in the secretion of the pro-inflammatory cytokines and chemokines, which trigger the inflammatory reactions.[17,19]

TLR2 is expressed on the cell membrane of monocytes, macrophages, myeloid DCs, endothelial cells, epithelial cells and nervous cells, and binds to various ligands including bacterial-derived lipoteichoic acid, lipopeptides and glycolipids, and fungal-derived beta glucan. Furthermore, TLR2 recognize a number of endogenous DAMP such as glycosaminoglycan, hyaluronan, hyaluronic acid, biglycan, snapin, heat shock proteins (such as HSP22, HSP60, HSP70, HSP90), high mobility group box protein 1 (HMGB1), surfactant protein A and D, eosinophil-derived-neurotoxin, gangliosides, versican, serum amyloid A and β-defensin-3.[20-22]

TLR2 is structurally expressed as a homodimer and heterodimer, in coalition with TLR1, TLR6 or TLR10 and thus recognizes a large variety of PAMPs.[23] The various types of leukocyte are directly influenced by

immunomodulatory impacts of TLR2 ligation such as B cells, $CD4^+$ and $CD8^+$ T cells, $\gamma\delta$ T- cells, NK cells, neutrophils, basophils and epithelial cells.[24,25]

It should be noted that the autoimmunity is the result of the activation of self-reactive lymphocytes and loss of self-immunologic tolerance. However, the improper TLR activation induced by self-constituents may cause non-infectious inflammation and autoimmunity.[16] In this review, we explain the involvement of the TLR2-related immunopathological responses in the development of MS and EAE diseases. The greater understanding of the TLR2-mediated pathways in the pathological mechanisms of MS and EAE may provide new insights to design the novel therapeutic agents. The targeting of TLR2 as a promising therapeutic potential for MS treatment could be considered in the future medical investigations.

TLR2-Linked Signaling Pathways

The first step in TLR2 signal transduction is the PAMP and/or DAMP-induced TLR2 homodimerization or heterodimerization (with TLR1, TLR6 and probably TLR10) that brings the TIR domains in their cytoplasmic regions into close adjacency, constructing a platform for signaling via TIR domain-containing adaptor proteins.[26]Myeloid differentiation primary response gene (MyD88), MyD88 adapter-like (MAL)/TIR domain-containing adaptor protein (TIRAP), TIR-domain-containing adapter-inducing interferon-β (TRIF), TRIF-related adaptor molecule (TRAM), and sterile α and armadillomotif containing protein (SARM) are the major TIR domain-containing proteins contributing in the intracellular transmission of signal.[27]

The TLR2-linked signal transduction principally depends on the adaptor proteins MyD88 and TIRAP/MAL.[26] Upon TLR2 ligation, both MyD88 and TIRAP/MAL are recruited through TIR–TIR interactions to the TLR2/TLR2, TLR2/TLR1, TLR2/TLR6 and probably TLR2/TLR10 heterodimers.[26] Following this process, MyD88 connects to interleukin-1 receptor-associated kinase (IRAK) complex, which comprises two active kinases (IRAK-1 and IRAK-4) and IRAK-2 and IRAK-3 subunits which are catalytically inactive.[28] Then the IRAK-4 phosphorylation activates IRAK-1 and subsequently recruit tumor necrosis factor receptor-associated factor 6 (TRAF-6) to construct a signaling complex of MyD88–IRAKs–TRAF-6 which activates a complex containing TGF-β-activated kinase 1 (TAK1), TAK1-binding protein 1 (TAB1), TAB2, and TAB3.[29] Activation of the TAK1/TAB complex elicits the mitogen-activated protein kinases (MAPKs) and the inhibitor of NF-κB kinase (IKK) complex.[30] IKK complex is composed of IKKα, IKKβ, and IKKγ/NEMO (NF-κB essential modulator).[31] Activation of IKK complex results in the phosphorylation of NF-κB inhibitor (IκB), which cause its ubiquitination and degradation.[32]Therefore, the NF-κB is released and translocated into cellular nucleus to start gene expression of pro-inflammatory parameters including IL-1β, IL-6, IL-8, IL-12, IL-17, TNF-α, IFN-γ, inducible nitric oxide synthase (iNOS) and ICAM-1.[33]

In another signal transduction pathway, the MyD88 is able to trigger the PI3K/AKT-linked signaling pathway, which could contribute in the production of an anti-inflammatory cytokine IL-10. The activation of PI3K/AKT pathway begins after recruitment of MAL (also known as TIRAP),whichisaMyD88-like adaptor protein.[34] In addition to the PI3K/AKT-mediatedanti-inflammatorypathway,TLR2ligationcan also induce Suppressor of cytokine signaling(SOCS) proteins which suppress signaling process, including MAPKs and Janus kinase-signal transducers and activators of transcription (JAK–STAT) pathways.[35]

TLR2-related signaling pathway also results in the consumption of IRAK1 and hence suppresses the formation of type I IFNs that is directed by other TLRs including TLR7 and TLR9. As TLR2 ligation reduces the synthesis of type I IFNs through reduction of IRAK1,[36] therefore, the TLR2-transmitted signal may interfere with signal transduction from other PRRs and following it may be affected by signals that are triggered from other immune-related receptors.

Expression of TLR2 and Its Ligands in CNS

The recognition of both exogenous PAMP and endogenous DAMP by TLRs may cause the stimulation of autoreactive lymphocytes in autoimmune diseases, such as MS. Indeed, a number of TLRs was linked with the development and progression of MS.[37] In MS disease, various kinds of leukocytes including monocytes, DCs, NK cells, lymphocytes ($CD4^+$ T, $CD8^+$ T and B cells) migrate into the CNS and cause myelin destruction, axon injury and neuronal cell death.[38] The residential cells and the infiltrated leukocytes into the CNS express a number of TLRs

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(such as TLR2) and their expression is enhanced in MS disease. Although, the raised TLRs expression may potentially involve in the pathogenesis of disease, they were also implicated in the neuroprotection process.[17,39] There are many evidences indicating the TLR ligands are able to induce CNS inflammation via the production of cytokines, nitric oxide and chemokines. However TLRs function in some conditions may be neuroprotective, if trigger in a suitable manner.[40]

In the CNS, TLR2 is expressed on the endothelial cells, microglia, astrocytes, oligodendrocytes and on infiltrated cells.[17,41] TLR2 plays a central role in the activation of glial cells and neuroinflammation, representing the importance of TLR2 in the pathogenesis of some neurological disorders.[21] TLR2 has been specified as a new player in CNS-related diseases such asMS,[42,43]Parkinson's disease.[44] and Alzheimer's disease.[45] In particular, the experimental and genetic evidences represent that TLR2 play a critical role in the MS pathogenesis as a result of its contribution in the neuroinflammatory responses that may lead to the death of neurons and tissue injury.[21,39] In addition, TLR2 is expressed on oligodendrocyte progenitor cells (OPC) and its activation suppresses OPC differentiation and myelination.[17]

Moreover, the MS patients exhibit high expression of TLR2 and its ligands in their mononuclear cells and in demyelinating regions in CNS.[43,46,47] Further, elevated levels of soluble TLR2 were indicated in the serum samples obtained from MS patients.[48]The high expression of TLR2 was also reported in the peripheral neutrophils in patients with RRMS.[49] The count of microglia and macrophages expressing the HMGB1, an endogenous TLR2 ligand, is increased in the RRMS.[50] Moreover, the elevated expression of HMGB1 was indicated in the peripheral blood mononuclear cells (PBMCs) and in the serum of RRMS and SPMS patients.[51,52] The elevated expression of HSP70,[53,54] versican,[55] serum amyloidA,[56] gangliosides[57] and biglycan[58] were also demonstrated in the PBMCs and/or in the CNS lesions of MS patients. Different DAMP are derived from various neuronal compartments following damage to the neurons[59] (Figure 1).

The contribution of the TLR2 was also reported in the EAE pathogenesis as an experimental model of MS.[60] Indeed, the TLR2-deficient mice exhibit a mild form of EAE. The low infiltration of T cell and microglia/macrophage also occur in the CNS of TLR2-deficient mice.[61] Even following the passive adoptive transfer of autoimmuneT cells, TLR2-deficient mice display the low infiltration of $CD4^+$ T cells in the brain as well as a milder EAE form.[61] The HMGB1 has been also found in damaged regions in EAE mice and its amounts correlate with inflammation scores.[50] Experimentally, in a mouse model of cuprizone-induced demyelination, it was found that the TLR2 expression was enhanced in the CNS in an area-related manner.[62] The activation of microglia and astrocytes was also attenuated in TLR2-deficient mice in an area-related manner.[62]

As mentioned, ligand-induced TLR2 dimerization recruits the MyD88 that in turn activates transcription factors, such as NF-κB and MAPKs, and finally lead to the production of pro-inflammatory cytokines.[33] The members of downstream of kinase (Dok) family act as modulators of protein tyrosine kinase(PTK) signaling.[15] It has been indicated that TLR2-related NF-κB activation and IL-6 secretion was exacerbated in astrocytes transfected withDok1-and Dok2-specificsmall interfering RNA (siRNA), representing that both Dok proteins attenuate the TLR2-related inflammatory signaling in astrocytes.[63] In contrast, silencing of the Dok1 expression diminish the TLR2-induced NF-κB activation and IL-6 production in microglia, while Dok2-specific siRNA unable to affect TLR2-related signaling and subsequent cytokine production in microglia.[63] Therefore, the TLR2-related signaling is controlled by a number of regulating molecules such as Dok1 and Dok2. The regulating molecules may perform distinct roles in different CNS resident cells. Hence. Dok1 and Dok2 differentially regulate TLR2-related signaling pathways in microglia and astrocytes. Therefore, Dok1 and Dok2 may be novel therapeutic targets in diseases that the microglial and astrocytic activation trigger excessive inflammatory reactions.

TLR2-Related Immunopathological Responses in MS and EAE

TLR2 Acts as a Linker between Infections and MS/EAE

There are accumulating evidences regarding a correlation between the several viral infections, such as EBV (64) and human herpes virus 6[22] and a number of bacterial infections, such as Chlamydia pneumoniae[65]

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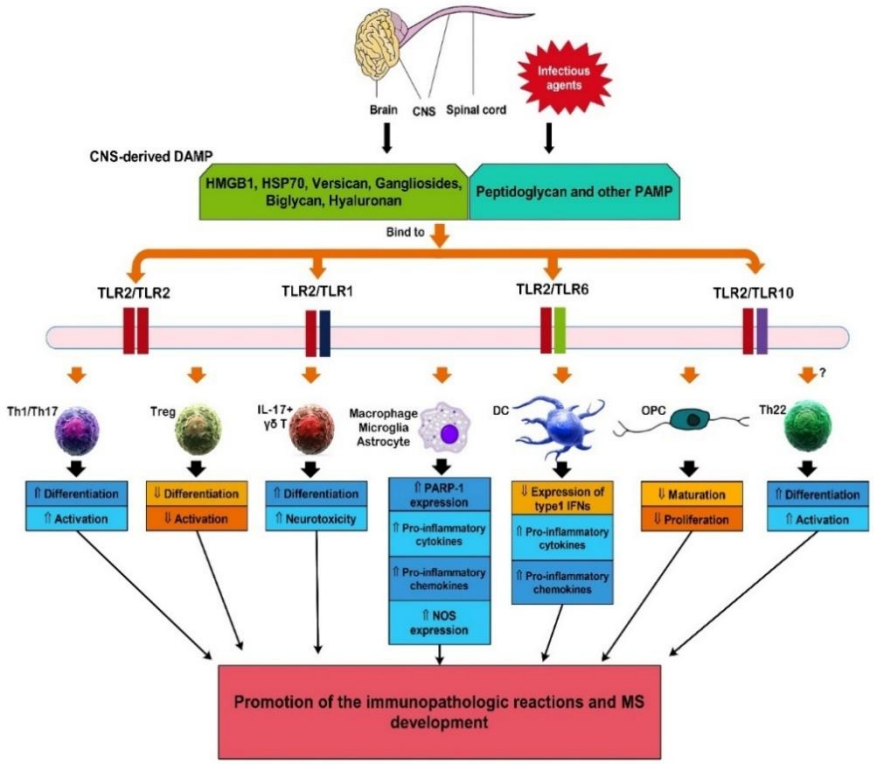

Figure 1. A schematic figure showing the involvement of the Toll-like receptor 2 (TLR2)-related pathways in the pathogenesis of multiple sclerosis (MS). Some central nervous system (CNS)-derived components [including high mobility group box protein 1 (HMGB1), heat shock protein 70 (HSP70), versican, gangliosides, biglycan, hyaluronan and serum amyloid A] and a number of pathogen-derived molecules (such as peptidoglycan) are recognized by TLR2/TLR2 homodimer and TLR2/TLR1, TLR2/TLR6 and TLR2/TLR10 heterodimers which are expressed by a number of infiltrated leukocytes and residential cells within the CNS. The TLR2-related signaling in lymphoid and non-lymphoid cells result in the induction of inflammatory responses during MS. TLR2 may contribute in the pathogenesis of MS through linking of infection with the MS, upregulation of Th1/Th17 cells, downregulation of regulatory T (Treg) cells, induction of interleukin-17 positive γδ T cells (IL-17$^+$ γδ T cells), inhibition of oligodendrocyte maturation, induction a Poly ADP-ribose polymerase-1 (PARP1)-dependent pathway in microglia, macrophages and astrocytes, induction of inducible nitric oxide synthase (iNOS), inhibition of type I interferons expression and induction of the pro-inflammatory cytokines and chemokines.

and group A streptococcus[66] with the development of MS. Exacerbation of MS following the active immunization with some vaccines, such as pneumococcal vaccine was also reported.[67] The infections may influence the susceptibility and the clinical patterns of MS. The bacterial infections frequently occur in MS patients and the relapsing form of MS has been reported during these infections.[68,69] Monocyte-derived dendritic cells (MDDCs) from MS patients with bacterial infections express more amounts of HLA-DR and costimulatory molecules than non-infected patients and produce higher amounts of IL-12, IL-17 and INF-γ.[70] Therefore, the microbial products can influence the MDDCs ability in order to increase autoreactive T-cell activation, which may be a reason for MS relapsing during bacterial infections. Some evidences indicate that TLR2 may operate as a linker between infectious agents and MS. The presence of several bacterial-derived TLR2 ligands were demonstrated in the brain and in the CSF of MS patients. For example, the peptidoglycan, a major component of the gram-positive bacterial cell walls, was observed in the brain of MS patients and within activated macrophage/DCs that express high amounts of surface co-stimulatory molecules (CD80, CD86 and CD40) which secrete high concentrations of pro-inflammatory cytokines such as IL-1α, IL-6, IL-12, TNF-α and INF-γ (71). The induction of TLR2 during infection may cause pathogen clearance by enhancing Th1/Th17 cell-related responses and decreasing Treg

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cell activity that may essentially contribute in the autoimmune-mediated tissue damage.[42]

Interestingly, the bacterial peptidoglycan is able to contribute in the inflammatory process of EAE diseases in the absence an active bacterial infection.[72,73] Peptidoglycan may be applicable as a replacement of heat killed Mycobacteria tuberculosis in complete Freund's adjuvant(CFA) for the EAE induction.[73] Moreover, the persistence of TLR2 ligands in the CNS was associated with low local expression of major peptidoglycan-degrading enzymes, including lysozyme and N-acetylmuramyl-l-alanine amidase.[72] The infection with *Streptococcus pneumoniae* worse the EAE severity in a TLR2-linked process.[74] Similarly, phosphorylated dihydroceramides from the common human oral bacterium *Porphyromonas gingivalis* induce IL-6 production from DCs, decrease spinal cord Treg cells and enhance the EAE severity in a TLR2-related manner.[75] These findings represent that the TLR2 induction by infectious stimuli can exacerbate MS, through down-regulating of Treg cell function. Therefore, it seems that the treatment of the MS-related infections may ameliorate the disease severity and diminish its clinical symptoms.

TLR2 Mediates Reinforcement of the Th1/Th17 Cell Responses

After the specific antigenic stimulation, different effector $CD4^+$ T cell subsets are differentiated from naïve $CD4^+$ T lymphocytes such as Th1, Th2, Th17 or Treg cells, which secrete exclusive cytokine patterns.[76] The differentiation of the effector Th1 lymphocytes from naïve $CD4^+$ T cells is regulated by cytokines IFN-γ and IL-12, which are synthesized by the DCs and NK cells, respectively.[77] Th1 cells release IFN-γ, which triggers the cell-mediated immunity and play prominent roles in defense against intracellular pathogens, anti-tumor immunity and development of some autoimmune disorders.[78] T-bet (T box expressed in T cells) has been identified as a principle transcription factor of Th1 cells.[77,79]

The differentiation of Th17 cells from naïve $CD4^+$ T lymphocytes is induced by some cytokines (especially IL-6 and TGF-β) and transcription factors retinoic acid receptor-related orphan receptors (RORγt and RORα).[79-81]Th17 cells secrete a number of cytokines, especially IL-17 (also called IL-17A), IL-17F, IL-21, IL-22, TNF-α and GM-CSF.[80,82]Th17 cells perform a major role in the defence against the various extracellular pathogens and involve in the inflammation process and autoimmune disorders.[81,83]

It should be noted that both MS and EAE are Th1/Th17 cell-mediated autoimmune diseases.[9,84,85] The pathogenic autoreactive Th1- and Th17 cells are infiltrated into the CNS of MS patients and EAE mice.[11,86]The Th1- and Th17 cells play a complementary function in the immunopathological process of MS and EAE.[84-86] Th1 cells conduct the macrophage accumulation in the spinal cord, while Th17 cells increase the neutrophils aggregation, especially in the brain.[86,87] The deficient mice in either RORγt or T-bet were resistant to EAE.[88,89]

Th1 cells perform a central role in promoting of the MS immunopathology by secreting IFN-γ.[90] The myelin basic protein (MPB)-specific Th cells isolated from MS patients mainly produce Th1 type cytokines, such as IL-2 and IFN-γ.[11,91] Moreover, the elevated IFN-γ and TNF-α levels in MS patients confirm that Th1 cells are pathogenic.[92,93] Treatment of MS patients with IFN-γ exacerbate the disease, whereas administration of monoclonal neutralizing antibodies against IFN-γ prevents MS attacks.[11,91]

The contribution of Th17 cells in immunopathogenesis of MS may perform through the recruitment of neutrophils into the CNS, induction of the reactive oxygen species (ROS) generation in brain endothelial cells, stimulation of microglia cells in order to produce the pro-inflammatory parameters, and the stimulation of astrocytes for producing CXC chemokines.[84,85] Some Th17 cell-derived cytokines (such as TNF-α) trigger the matrix metalloproteinases expression which play a critical role in the degeneration of brain blood barrier (BBB).[94,95] The high expression of matrix metalloproteinases (including MMP-2, MMP3, MMP-7 and MMP-9) has been shown in the CNS of patients with MS.[95] The increased formation of a Th17 cell-related chemokine (CCL20) and diminished production of a Th2/Treg cell-related chemokine CCL22 were also indicated in MS patients.[96,97]

TLR2 Ligation also causes the expression of IL-1, IL-6 and IL-12, which play a principle role in the differentiation of naïve $CD4^+$ T lymphocytes into Th1 and Th17 cells.[17]The endogenous TLR2 ligands may have an increasing impact on the differentiation, function and maintenance of Th1- and Th17 cells. TLR2 stimulation promotes the Th17 cell

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differentiation and proliferation, which can lead to the more Th17 cell-related cytokine production.[98]

It has been indicated that the expression of TLR2, TLR4 and TLR9 on $CD4^+$ and $CD8^+$ T cells in patients with MS were higher than the healthy individuals. The stimulation of purified CD4+ and CD8+ T cells from MS patients with a TLR2(Pam3Csk4) cause the higher formation of pro-inflammatory cytokines compared to the activation with TLR4 or TLR9 ligands.[99] Further, the amounts of IL-6, IFN-γ, IL-17 and GM-CSF induced by Pam3Csk4-activated $CD4^+$ T cells were directly associated with the severity of disease. A similar correlation was observed between the amounts of IL-17 production by Pam3Csk4-induced $CD8^+$ T cells, and clinical parameters.[99]

As mentioned previously, the elevated expression of HMGB1 was demonstrated in the PBMCs of RRMS and SPMS patients.[51] HMGB1 increases the polarization of Th17 cells via up-regulation of the production of TLR2 and IL-23 in monocytes from rheumatoid arthritis patients.[100] Furthermore, induction of TLR2 by HMGB1 in monocytes from patients with ischemic stroke increase the Th17 cell-related response.[101] TLR2 also promotes Th17 cell-related responses in hepatitis B virus infection.[102] Similar events may happen in patients with MS (Figure 1).

Using an EAE model, it was demonstrated that TLR2 potentiate the Th17 cell-mediated autoimmunity and the loss of TLR2 in $CD4^+$ T cells can markedly ameliorate the EAE.[61] Moreover, TLR2 stimulation increases EAE development and promotes the clinical symptoms. Further, TLR2-deficient mice display low Th17 cell-related responses and a reduction in the EAE symptoms.[61]In another study on a murine model, it was also indicated that the TLR2 signaling promotes the IFN-γ, IL-6 and IL-17 secretion, causing the differentiation of Th1- and Th17 cells that may lead to EAE development.[103] In EAE, the TLR2-mediated signaling was associated with a low number of central $CD62L^+$ Treg cells and high infiltration of IL17-producing $CD4^+$ T cells into the CNS. Moreover, TLR2-deficient mice exhibit fewer IL17-secreting $CD4^+$ T cells and high proportions of central $CD62L^+$ Treg cells in the CNS.[104]

TLR2 Mediates Impairment of Treg Cell Responses

Treg cells constitute approximately 5–15% of the peripheral $CD4^+$ T cells sort into two major subsets, including natural Treg (nTreg) and inducible Treg (iTreg) cells. The nTreg subset is developed from precursor cells in the thymus whereas iTreg cells arise from naïve $CD4^+$ T lymphocytes in the secondary lymphoid organs following antigenic recognition in the presence of IL-2 and TGF-β.[105,106] The Treg cell activity was controlled by a major transcription factor FOXP3, and the mutations of the foxp3 gene are able to impair the Treg cell activity, therefore it can be led to the development of various types of autoimmune disorders.[106,109]

The Treg cell-related immunosuppressive cytokines were identified as IL-10, TGF-β and IL-35, which play an essential role in inhibiting CNS-related autoimmune diseases.[17,107,108] Recent investigations reveal that he number and/or function of Th17 cells were increased, while the frequency and/or immunosuppressive function of Treg cells were diminished in MS patients,[83,106] representing that the modification of the Th17/Treg cell balance contributes to the development of disease. Indeed, the separated Treg cells from MS patients display low inhibitory effects on T cell expansion following the specific antigenic stimulation with myelin-derived components. Moreover, Treg cells isolated in relapse phase of the MS exhibit weak suppressor function.[109] The functional defects in Treg cell function cause autoimmune response to neuronal myelin due to the activation of the self-reactive T cells.[109]

It was reported that the TLR2 has modulatory effects on the activity of human naïve and memory Treg cells. The ligation of TLR2 on myeloid dendritic cells (mDCs) results in the IL-23 synthesis that enhances the secretion of IL-17A from $CD4^+$ T cells.[110] The TLR2 stimulation with Pam3Cys reduces the immunosuppressive functions of Treg cells and induces a deviation fromTreg-toTh17cell-related responses in PBMCs isolated from healthy individuals.42 It was demonstrated that the Treg cells from patients with RRMS express higher amounts of TLR2 in comparison with healthy subjects. Naive and effector Treg cells from patients with RRMS are more vulnerable to TLR2-mediated inhibition of immunosuppressive activity and to Th17 cell development than in healthy individuals.[42]

Indeed, the elevated TLR2 expression by T cells from MS patients represent that these individuals may be more vulnerable than healthy individuals to the damping effects of microbial constituents on Treg cells.[42] These findings represent that the higher TLR2

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expression may be an important account for the low Treg cell activity in MS patients. The TLR2 ligation causes the deviation of the Treg/Th17 balance toward Th17 cell-related responses in patients with RRMS patients. Indeed, the TLR2 induction results in the IL-6 formation by $CD4^+$ T cells in RRMS patients.[42] In the presence of TGF-β, IL-6 promotes the generation of Th17 cell while preventing Treg cell differentiation, thereby regulating the balance between Th17- and Treg cells.[83] In particular, IL-6 is required for TLR2-induced inhibition of Treg cell-relate immunosuppressive activity as neutralization of IL-6 also abolish the TLR2-induced formation of IL-17 and IL-22 in human Treg cells.[111] In addition to its direct influences on Treg cells, IL-6 cause the unresponsiveness of effector T cells to Treg cell-mediated inhibition in MS patients.[112] The effect of TLR2 on Treg cell function is mediated through IL-6, therefore, the IL-6 neutralization may have therapeutic potential in MS as indicated in other immune-mediated diseases, including rheumatoid arthritis and a MS-related neuroinflammatory disease, neuromyelitisoptica.[113,114]

TLR2 Mediates Development of IL-17^+ γδ T Cells

The IL-17^+ γδ T cells and microglia, the major residing immune cells in the brain, are involved in several CNS-related disorders such as MS and EAE diseases.[115,116] There are evidences supporting that TLR2 may involve in the EAE development trough induction of the IL-17^+ γδ T cells. Similar TLR2-mediated IL-17^+ γδ T induction may occur in MS.

Elevated number of IL-17^+ γδ T cells was indicated in the brain of EAE.[117]In EAE, a pathogenic role of IL-17^+ γδ T cells was reported in the beginning of the disease.[115,118]The γδ T cell-deficient mice display an attenuated form of EAE disease and a delay in the appearance of symptoms.[115] Further, γδ T cells-derived IL-17 increases the secretion of IL-17 from $CD4^+$ T cells.[117] These observations represent that IL-17^+ γδ T cells involve in the pathogenesis of EAE through promotion of the harmful Th17 cell-related responses. It has also indicated that TLR2-deficient γδ T cells display diminished IL-17 production in response to IL-23.[61]

It was demonstrated that supernatants from mouse microglia stimulated with a TLR2 agonist (Pam3CysSK4) induce naïve γδ T cells to secrete IL-17. The stimulation of mouse microglia via a TLR2 agonist and the subsequent secretion of IL-23 and IL-1β play a principle role in development of IL-17^+ γδ T cells.[116]The IL-17^+ γδ T cells differentiated by supernatants from TLR2-activated microglia also display neurotoxic activity *in vitro*. The IL-17^+γδ T cell-mediated neurotoxicity require a direct cell-cell joining between effector T cells and neuronal cells.[116] These observations represent that microglia activation through TLR2 play an important role in polarization of γδ T cells towards neurotoxic IL-17^+ γδ T cells (Figure 1).

TLR2 Mediates Inhibition of Oligodendrocyte Maturation and Remyelination

The myelin is a substance that covers the axon of some neurons, enabling quick nerve conduction and increase axon integrity. The loss of myelin is named demyelination, which is a hallmark of a number of CNS-related disorders such as MS.[119] The myelin synthesis is done by oligodendrocytes that are differentiated from oligodendrocyte progenitor cells (OPC). After inflammatory injury, the remyelination is mediated byOPC that migrate and differentiate into oligodendrocytes.[120]

Hyaluronicacid (also called hyaluronan) is an anionic glycosaminoglycan polymer, which accumulates in demyelinated lesions in the brain white matter of MS patients. Demyelination areas in the CNS of MS patients and EAE mice also show high levels of hyaluronan (HA) accumulation, which is related to the impairment of remyelination.[121-123]Therefore, the impaired hyaluronan-mediated remyelination is an important phenomenon that occurs in MS patients and EAE mice.

HA is once digested by the hyaluronidases expressed by OPC, inhibit the maturation of OPC and remyelination via binding to TLR2.[17,123] It has been suggested that high molecular weight fragment of HA (HMW-HA) may be split by hyaluronidases into smaller components and then stimulate TLR2. Therefore, HA binds to TLR2, which also is known as a hyaluronan receptor and is predominantly expressed on the surface of astrocytes and OPC.[121] Thus, inhibition of the remyelination in MS is mediated through HA, partial HA degradation and TLR2 ligation on oligodendrocytes.[123] HA is involved in the regulation of remyelination by binding to TLR2, which inhibits the maturation of OPC and remyelination.[121] HA suppresses the maturation of

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the oligodendrocyte *in vitro*, in a dose dependent manner.[121]

The HMW-HA suppresses remyelination in a murine model of lysolecithin-induced demyelination, however, the TLR2 null mice exhibit more rapid and efficient remyelination.17.[17] Local micro-injection of zymosan (a TLR2 activator) into the rat spinal cord also elicits focal demyelination and prevents OPC proliferation, differentiation and remyelination.[124] These findings indicate that the agonists of TLR2 inhibit oligodendrocyte maturation, representing a TLR2-dependent inhibitory effect on oligodendrocyte maturation. In murine models, it was demonstrated that neutralizing antibodies to TLR2 block the effects of HA on oligodendrocyte maturation.[125] Therefore, inhibition of TLR2 may confer neuroprotection in brain injury.

TLR2 Mediates a PARP-1-Dependent Pathway in Microglia, Macrophages and Astrocytes

Poly ADP-ribosylation (PARylation) is one of the basic modifications of proteins, as polymers of ADP-ribose is attached to the glutamic acid, aspartic acid or lysine residues in the target proteins.[126] Poly ADP-ribose polymerase-1 (PARP-1) catalyzes the PARylation of some proteins such as histones, topoisomerases and DNA helicases resulting in the relaxation of the chromatin composition, protein-protein interaction and DNA-protein binding, therefore, leading to gene expression.[126] High PARP-1 activity was observed in the monocytes from patients with SPMS.[60] PARP-1-related pathway may contribute in the induction of inflammatory responses by promoting the expression of pro-inflammatory cytokines, adhesion molecules and the enzymes belonging to the oxidation-reduction system.[126,127] Therefore, PARP-1 may potentially play an essential role in the pathogenesis of inflammatory diseases.

In the CNS, TLR2 may contribute in the neuroinflammation through a PARP-1-associated pathway as indicated in a progressive EAE model.[60]Higher serum concentrations of 15-α-hydroxicholestene (15-HC) were observed in patients with SPMS and in mice with secondary progressive EAE. The administration of 15-HC worse the EAE severity via a PARP-1-and TLR2-dependent process.[60]15-HC activate microglia, macrophages and astrocytes, and enhance the expression of TNF-α, iNOS and CCL2 in CNS-infiltrating monocytes/macrophages, through a pathway involving TLR2 and PARP-1.[60] The inhibition of PARP-1 prevents the progression of EAE.[60] Experimentally, it has been demonstrated that PARP-1 or PARP-2inhibitors reduce the migration of DCs into CNS, suppress the encephalitogenic response and reduce the infiltration of the Th1 and Th17 cell in the CNS during EAE.[128,129] Thus, theTLR2-PARP-1 pathway may be a potential new therapeutic target in MS.

TLR2 Mediates the Inhibition of Type I Interferons Production

It was demonstrated that the production of type I interferons (IFNs) was impaired in patients with MS.[130] The recombinant IFN-β is an effective first-line therapy against MS.[131] IFN-β inhibits$CD4^+$naïveT cell differentiation into Th1/Th17 cells, suppresses the secretion of the Th17 cell-polarizing cytokines IL-1β and IL-23 by human DCs, stimulates Th2 cell-polarizing cytokines, limits leukocyte migration across the BBB and increases neuronal survival.[131,131]

In humans, DCs were classified into two major subsets, including plasmacytoid dendritic cells (pDCs) and myeloid dendritic cells (mDCs). The pDCs quickly release type I IFNs after induction through binding of pathogen-derived nucleic acids to TLR7 and TLR9.[131] It was showed, that pDCs are composed of two different subsets.[132] Type 1 pDCs (pDC1), expressing high extent of CD123, low extent of CD86 and TLR2, are the major producers of IFN-α and are the inducers of IL-10-secreting Treg cells. On the other hand, type 2 pDCs (pDC2) expressing low extent of CD123, and high extent of CD86 and TLR2, are the major sources of IL-6 and TNF-α that play a prominent role in the differentiation of naïve T cells toward Th17 cells.[133] Interestingly, the ratio of pDC1/pDC2 in MS patients is deviated toward pDC2 as compared with healthy individuals, representing a more susceptibility to develop Th17 cell-related inflammatory responses in these individuals.[131,132] IFN-β treatment restores the aforementioned imbalance in the pDC1/pDC2 ratio in MS patients and IFN-β-induced changes in DCs-derived cytokines suppress the differentiation of Th17 cells.[131]

Mice with deficient in IFN-β, IFNs receptor or its downstream related signaling molecules also display an increased susceptibility to EAE, indicating that defective endogenous IFN-β production or its-related signaling leads to a higher vulnerability for EAE.[134]

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The TLR2-related molecular mechanisms, which are responsible for the reducing type I IFNs production, may be performed through depletion of a signaling factor IRAK1. IRAK1 is an essential factor for TLRs-induced type I IFN expression.[135] It was indicated that signal transmission from TLR2 results in the reduction of IRAK1 and therefore suppresses the expression of type I IFNs directed by other TLRs such as TLR7 and TLR9. The results of a study revealed that the levels of IRAK1 were reduced quickly after treatment with TLR2 agonist.[36]It seems that TLR2 ligation down-regulates the formation of type I IFNs via reduction of IRAK1 which may play a critical role in the development of MS (Figure 1).

Protective Role of TLR2 in MS

There are many investigations regarding the contribution of the TLR2 in the pathogenesis of MS, however, the results of a number of studies suggest a protective role for TLR2 against MS. It has been reported that the serum levels of Lipid 654 [(L654), a microbiome-derived molecule, which acts as a TLR2 ligand] in MS patients was lower than healthy individuals.

Moreover, the formation of the small heat shock protein alpha B-crystallin (HSPB5) is enhanced by stress-exposed oligodendrocytes in MS patients. The HSPB5 levels may increase up to 20 fold during MS disease.[136]HSPB5 prevents neuronal and glial cell apoptosis, diminishes inflammation, decreases tissue damages and enhances recovery in a number of animal models of neuroinflammatory disorders.[136,137] HSPB5 exerts its beneficial effects in part through the induction TLR2-mediated anti-inflammatory, neuroprotective and tolerogenic responses in microglia and macrophages.[137] HSPB5 may induce the expression of IL-10, indoleamine-2,3-dioxygenase-1 and TGF-β, strongly represent the induction of anti-inflammatory responses through a TLR2-mediated process.[137] This protection terminates by IFN-γ, which is produced within the CNS during inflammatory demyelinating diseases.[138] It was also indicated that the neuroprotective effects of HSPB5 might perform through the induction of immune-modulating enzyme cyclooxygenase-2 (COX-2) in microglia.[139]

Moreover, the TLR2 expression on B cells and DCs is increased in helminth-infected MS patients, who display better clinical symptoms than uninfected patients.[140] This improvement was associated with Treg cell induction and increased levels of TGF-β and IL-10 and decreased levels of IFN-γ, IL-12, and IL-17.[140] The induction of TLR2 on human APC such as DCs and B cells by helminths regulates their cytokine profile toward an anti-inflammatory response.

H. pylori also induces powerful Treg cell responses and weak Th1 cell responses through TLR2 induction.[15]The *H. pylori*-induced activation of Treg cells downregulate inflammatory responses and contribute to the bacterium persistence in asymptomatic *H. pylori-infected* individuals.[15,141] Therefore, *H. pylori* infection has been also considered potentially protective against MS.[142,143]

There are also evidences showing that the TLR2 stimulation induces Th2 cell-linked responses. The results from a number of investigations indicating that Th2 cells may play a protective role against MS. Therefore, it has been also postulated that TLR2 induction may be protective against MS through recruitment of Th2 cells.[144] The zymosan as a TLR2 ligand may also reduce the severity of MS by inducing peripheral blood DCs from MS patients to produce IL-10, which suppresses IL-23 and IL-1β secretion.[17]

Intestinal commensal bacteria also confer protection against CNS demyelination and inflammation during EAE through a TLR2-mediated pathway.[145] Further, the administration of low doses of TLR2 ligands (including $Pam2CSK_4$and L654) in a model of EAE induces TLR2-related tolerance and attenuates disease. The EAE amelioration was related with reduced macrophage activation and diminished Th17 cells within the CNS, and elevation in splenic Treg cells.[146]Experimentally, it has also been indicated that the stimulation of microglia with a TLR2 agonist, Pam2CSK4, is neuroprotective due to the induction of alternative (M2 type) microglial activation after laser-induced spinal cord injury.[147] It was also indicated that stimulation of microglia using TLR2, TLR4, and TLR9 agonists result in the production of anti-inflammatory cytokine IL-10.[148]

The reasons for aforementioned TLR2-related plasticity in MS and EAE diseases remain to be clarified in future investigations. A considerable plasticity reported in the TLR2-associated recognition and signaling pathways, may be due to the variations in the composition of the PAMP molecules that act as TLR2 ligands. For instance, upon stimulation with staphylococcal-derived peptidoglycan, PBMCs

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produce IL-10 via a TLR2-dependent mechanism that reduce the T cell response to staphylococcal-related superantigens.[149] Additionally, LPS from some bacterial species induces inflammatory responses via a TLR2-mediated mechanism, while the LPS from other species does not induce pro-inflammatory cytokine expression.[22] Furthermore, the expression of some innate immunity-related receptors, including CD14 and CD36 may interfere with the TLR2-related signaling pathways. The expression of these molecules is essential for the induction of TLR2-related inflammatory responses, but is not necessary for the production of anti-inflammatory cytokine IL-10.[150]

The signals originating from other PRRs may interfere with the TLR2-mediated inflammatory reactions in an agonistic or antagonistic process. Collectively, the type, dose and composition of PAMP or DAMP, the type of cell expressing TLR2, signal coming from other PRR, the presence of other inflammatory mediators in the microenvironment and the TLR2 gene polymorphisms may influence the nature and magnitude of TLR2-related responses.

The Targeting of TLR2 as APotential Therapeutic Approach of MS

The evidences provided here indicate a pathologic or protective role for TLR2 during MS disease, however, the evidences regarding the pathologic role of receptor are clearly more than of its protective role. Therefore, TLR2 may be a favorable target for the control of inflammation in MS disease, although it seems much time has remained until that time. It has indicated that in vitro culture of monocyte with glatiramer acetate, an immunomodulating approved drug for treatment of MS, suppresses the TLR2-induced cytokine production such as TNF-α.[151]

The TLRs-related responses may be modulated in three main areas including intervention in the ligand-TLR interaction, targeting of downstream signaling molecules or regulating the receptor expression. Structurally, the extracellular domain of the TLRs contains leucine rich repeat (LRR), which may be considered as a target of agonist or antagonist drugs.[152] Therefore, the regulation of TLR2-associated immune responses by using specific agonists or antagonists may lead to the reduction of the undesirable side effects or reinforcement of the desirable beneficial effects. It should be noted that there are some controversies regarding the induction of TLR2 on the effector T cell functions. It was reported that TLR2 agonists induce the expansion of the both $CD4^+$ Treg and $CD8^+$ Treg lymphocytes in PBMCs isolated from asthmatic patients during immunotherapy program.[153] On the other hand, it was indicated that TLR2 ligation with Pam3CSK4 (a synthetic triacylated lipopeptide that acts as a TLR2 agonist) reduces the suppressive functions of Treg cells.[111] However, the immunomodulatory properties of Pam3CSK4 were indicated on the cytokine secretion by lymphocyte collected from patients with infection or inflammatory diseases.[154,155] The reasons for these controversies remain to be clear in more studies. The staphylococcal superantigen-like protein 3 (as an example of TLR2 antagonist) inhibits TLR2-related immune responses via blocking of ligand binding and preventing TLR2-related downstream signaling.[156] The therapeutic potential of TLR2 agonists or antagonists concerning MS disease needs to be considered in future investigations.

There is no licensed specific TLR2 inhibitor for using in human, yet. A small molecule $C_{16}H_{15}NO_4$ (C29) and its derivative, *ortho*-vanillin (as potential TLR2-specific inhibitors) act by specific binding to a particular pocket located inside the TIR domain of TLR2, altering its activity and conformation.[157]The human embryonic kidney 293 (HEK293) and human acute monocytic leukemia (THP-1) cells transfected with TLR2 exhibit low level secretion of IL-8 after treatment with C29.

Down-regulation of the expression of TLR-related cytokine and chemokine response also was indicated by using soluble decoy receptors. A soluble kind of TLR2 (sTLR2) binds to a vast board of related PAMP and DAMP, hence, prevents the ligation of receptor on cellular membrane.[158]Low amounts of sTLR2 were indicated in a number of infectious and inflammatory diseases.[158-160] The possible using of sTLR2 for attenuating MS disease need more consideration.

The suppression of the TLRs-related responses with neutralizing monoclonal antibodies may be a promising therapeutic strategy. A specific monoclonal antibody against TLR2 reduces the formation of the pro-inflammatory cytokines in *Malasseziafurfur*-infected keratinocytes as an experimental model of psoriasis.[161]

Further, the administration of the TLR2-specific monoclonal antibody may has beneficial therapeutic

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effects. OPN-305 is a humanized IgG4 monoclonal that binds to the ligand-binding site of TLR2, suppressing its heterodimerization with TLR1 or TLR6. OPN-305 inhibits the TLR2-associated cytokine secretion.[162] Now, the efficacy and safety of OPN-305 in delayed graft function are evaluated in a phase-II of clinical trial study (NCT01794663).[163] A single-chain variable fragment (scFv) against TLR2 also suppresses the TLR2-related immune responses *in vitro*.[164] Potentially, similar antibody or fragment may be developed for modulating inflammatory reactions in MS.

There are reports indicating that TLR2 agonists may contribute to the prevention of EAE (165). The TLR2-induced expression of IL-12 and TNF-α was also reduced in mice administrated C29.[157] Moreover, a TLR2 peptide (TLR2-p) inhibits the TLR2 dimerization and decreases the extracellular signal-regulated kinases (ERK) expression and the expression of the pro-inflammatory cytokines.[166] In an experimental model of colitis, it was indicated that TLR2-p reduces the production of the pro-inflammatory cytokines and result in the attenuation of disease.[166] Mice treated with a neutralizing antibody against TLR2 were protected against sepsis-related death due to infection with *Bacillus subtilis*.[167]

The TLR-related regulatory proteins may have also therapeutic potentials. A20 is a powerful anti-inflammatory molecule that prevents NF-κB activation in upstream regions by direct suppression of IKK-inducing proteins such as TRAF6.[168]TheA20expression is decreased in the peripheral blood samples from MSpatients in comparison with healthy individuals.[169] The A20-deficient mice are more susceptible to EAE induction and the A20-deficient microglia also display more pro-inflammatory characteristics such as IL-1β production.[170]The TLR2-related responses are also influenced by the Pellino family proteins (including Pellino1, Pellino2 and Pellino3) which bind to downstream TLR-associated signaling molecules IRAK1, IRAK4 and TRAF6 therefore, perform a key role in the regulating of TLR signaling.[171] The Pellino1 and Pellino2 have pro-inflammatory effects, while Pellino3 exert a negative regulatory role.[172] It has been indicated that the Pellino1 expression is increased in the microglia cells from EAE mice that mediates the expression of the proinflammatory elements in microglia and reinforces the recruitment of T cells into the CNS.[173] The severity of EAE is reduced in Pellino1-deficient mice.[173] The low expression of Pellino3 together with the high expression of Pellino1 and Pellino 2 may be important parameters influencing the severity of MS.

The Dok1 and Dok2 regulators also control the TLR2 activity. The transmitted signals from TLR2 elicit the Dok1 and Dok2 phosphorylation. The phosphorylated Doks inhibit ERK and NF-κB in the TLR2-associated pathways.[63] Further, IRAK-M binds to IRAK-1/IRAK-4 connected to MyD88, thereby inhibiting IRAK-1/TRAF6 downstream signaling.[174] In addition, Tollip serves as a suppressor of NFκB through direct binding to IRAK-1 and IRAK-2 and preventing their auto-phosphorylation.[175] Syk is also directly associated with several TLR-related signaling components, including MyD88, TRIF, TRAF3, TRAF6 and TAK1.[176] Syk exerts its anti-inflammatory effects through suppression of TRAF6 in the MyD88-dependent pathway.[176] The elucidation of the exact role of Pellino family proteins, Dok molecules, IRAK-M, Tollip and Syk during MS development and their therapeutic potentials need more considerations (Figure 2).

Eventually, reducing the TLR2 expression may provide another strategy to regulate the inflammatory responses resulted by TLR2. One possible method for this purpose is the using of microRNAs (miRNAs) or siRNA. The reducing effects of a TLR2-specific siRNA on the corneal inflammation and attenuation of keratitis were reported in a rat model.[177] The miRNA-21 also suppresses the TLR2-associated lung inflammation in mice.[178] Similar strategies by targeting TLR2-related signaling pathways may have therapeutic potential capacities to consider in future investigations concerning the treatment of MS.

Our study may have some limitations that need to consider in future studies. The possible influences of the TLR2-related signaling on other effector cells that are involved in the pathogenesis of the MS and EAE (CTLs, Th9-, Th22-, B-, NK-, and NKT cells) need to be clarified in further researches. Further, evaluation of the genetic variations such as single-nucleotide polymorphism (SNP) in the TLR2 gene and its related signaling molecules and their association with susceptibility to MS need more consideration.

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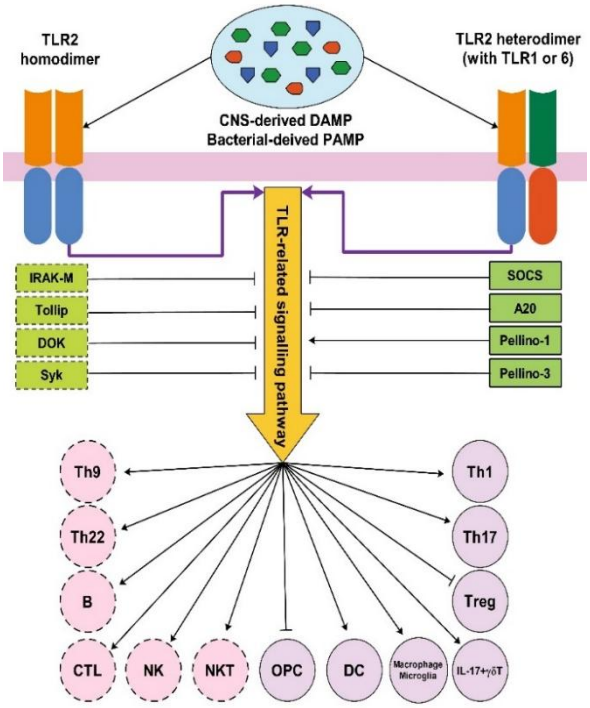

Figure 2. A schematic figure showing the influences of the regulatory proteins on the Toll-like receptor 2 (TLR2)-related pathways. The central nervous system (CNS)-derived components and pathogen-derived molecules are recognized by TLR2 and then trigger the TLR-related signaling pathway, which influence the infiltrated leukocytes and residential cells of the CNS. The regulatory proteins also affect the TLR-mediated responses. The roles of parameters displayed in discontinuous shapes, which either affect or are affected by TLR2-related signaling, remain to be clarified in further researches on both multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE) diseases.

CONCLUSION

Although there are considerable similarities between EAE and MS, however, there are some differences between the two disorders such as the disease induction, course of disease progression, localization of the CNS damages, and participating immunopathological mechanisms.[179] For example, EAE and MS are mainly dominated by $CD4^+$ T- and $CD8^+$ T cells, respectively.[179] Father, IFN-γ is not required for EAE induction and may have may have preventive effects, while IFN-γ supplementation exacerbate MS.[179] As mentioned, there are considerable overlap between MS and EAE regarding the TLR2-related immunoplathological mechanisms. However, the differential roles of TLR2 concerning the aforementioned differences between EAE and MS need to be elucidated in future studies.

The expression of TLR2 is up-regulated in residential and infiltrating cells during MS and EAE. Some of DAMP act as TLR2 ligands and it is possible that harmful stimuli such as injury, infection, stress and cell death trigger DAMP releasing within the CNS. The available evidences indicate that the pathologic roles of TLR2 are clearly more than of its protective role during MS and EAE diseases. The binding of DAMP and/or PAMP to TLR2 may contribute a significant role in the development of MS and EAE through promotion of the chronic inflammation and demyelination within the CNS. The inhibition of abnormal TLR2-related signaling pathways and its linked inflammatory responses may have a potential therapeutic approach for the treatment of MS disease. However, the results of a few studies indicate that TLR2 may have a protective role against EAE and MS diseases. The factors influencing the plasticity of TLR2-dependent responses during MS and EAE diseases and modification of this plasticity in a favorable direction should also be considered in further investigations.

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