Life Sciences 238 (2019) 116924

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Review article

High mobility group box 1 (HMGB1) protein in Multiple Sclerosis (MS): Mechanisms and therapeutic potential

Yam Nath Paudel[a,*], Efthalia Angelopoulou[b], Bhuvan K C[c], Christina Piperi[b,***], Iekhsan Othman[a,**]

[a] Neuropharmacology Research Laboratory, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway, Selangor, Malaysia
[b] Department of Biological Chemistry, Medical School, National and Kapodistrian University of Athens, Athens, Greece
[c] School of Pharmacy, Monash University Malaysia, Jalan Lagoon Selatan, 47500, Bandar Sunway, Selangor Darul Ehsan, Malaysia

ARTICLE INFO

Keywords:
HMGB1
Multiple sclerosis
Inflammation
Experimental autoimmune encephalomyelitis
Demyelination

ABSTRACT

Multiple sclerosis (MS) is an autoimmune chronic inflammatory disease with distinctive features of focal demyelination, axonal loss, activation of glial cells, and immune cells infiltration. The precise molecular mechanism underlying the disease progression remains enigmatic despite of the rapid progression on experimental and clinical MS research. The focus of MS therapy relies on the repression of the pathogenic autoimmune response without compromising an adaptive immune response. High mobility group box-1 (HMGB1) protein is a ubiquitous nuclear protein driving pro-inflammatory responses as well as targeting innate immune signaling that initiates and mediates autoimmunity as well as sterile injury. A considerable amount of experimental and human studies suggests the contribution of HMGB1 in the pathogenesis of MS/experimental autoimmune encephalitis (EAE). In this regard, HMGB1 protein has gained increased attention, as an emerging possible therapeutic target against MS. This is more strengthened by the promising therapeutic outcome demonstrated by HMGB1 neutralizing agents in the experimental EAE model. Herein, we attempt to shed more light on the molecular crosstalk of HMGB1 protein in the pathogenesis of MS/EAE suggesting that HMGB1 blockade could impede the pro-inflammatory loop that drives MS autoimmunity.

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disorder of the central nervous system (CNS) that might lead to the progressive neuro-axonal degeneration [1]. MS might cause injury to myelin sheaths, oligodendrocytes as well as to the axons and nerve cells itself [2–5]. MS exhibits complex pathophysiology involving several cell types, myelin-associated autoantigens as well as modifying genetic and environmental contributors [6]. MS symptoms normally vary depending on the plaque's location within the CNS [7]. Focal demyelination that is observed in white and grey matter (GM) of the brain and the spinal cord, denotes the loss of myelin sheaths which are the key pathological hallmarks of the disease. Notably, these plaques or lesions are also featured by inflammation, gliosis, oligodendrocyte and neuronal loss [8]. Despite the rapid progression in MS research over the decades, the underlying pathogenesis of the disease is still not well understood. MS is an autoimmune disease with its initiation and progression stages being mainly dependent on the autoimmune response against myelin antigens. Moreover, genetic susceptibility and environmental triggers are believed to contribute to the initiation of the disease [6]. The global prevalence of MS has been reported to affect around 2.3 million of people worldwide [9] with women presenting a two-fold higher risk than men [10]. Despite its relatively low prevalence, MS has gained increased attention due to its high frequency in people between 30-40 years affecting their reproductive age and contributing to a negative social and economic impact [11,12].

* Corresponding author. Neuropharmacology Research Laboratory, Jeffrey Cheah School of Medicine and Health Sciences, MONASH University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, Selangor, Malaysia.
** Corresponding author. Neuropharmacology Research Laboratory, Jeffrey Cheah School of Medicine and Health Sciences, MONASH University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, Selangor, Malaysia.
*** Corresponding author. Department of Biological Chemistry, Medical School, National and Kapodistrian University of Athens, 75 M. Asias Street, 11527, Athens, Greece.
E-mail addresses: yam.paudel@monash.edu (Y.N. Paudel), cpiperi@med.uoa.gr (C. Piperi), ",0,0,2
>Iekhsan.othman@monash.edu (I. Othman).

https://doi.org/10.1016/j.lfs.2019.116924
Received 16 July 2019; Received in revised form 27 September 2019; Accepted 29 September 2019
Available online 10 October 2019

Abbreviations

MS	Multiple sclerosis
EAE	Experimental autoimmune encephalomyelitis
HMGB1	High mobility group box 1
Anti-HMGB1 mAb	Anti-HMGB1 monoclonal antibody
DAMP	Damage-associated molecular patterns
PRRs	Pathogen recognition receptors
TLRs	Toll-like receptors
RAGE	Receptor for advanced glycation end products
BBB	Blood-brain barrier
CNS	Central nervous system
CSF	Cerebrospinal fluid
MOG	Myelin oligodendrocyte glycoprotein
DCs	Dendritic cells
IFN	Interferon
ICV	Intracerebroventricular
GM	Grey matter
GFAP	Glial fibrillary acidic protein
IL	Interleukin
PBMC	Peripheral blood mononuclear cells
PPMS	Primary progressive multiple sclerosis
SPMS	Secondary progressive multiple sclerosis
RRMS	Relapsing-remitting multiple sclerosis
DMT	Disease-modifying treatment
TGF-β	Transforming growth factor-β
NF-κβ	Nuclear factor κ light chain enhancer of activated β cells
TNF-α	Tumour necrosis factor-α
Th1	T helper 1 cells
VCAM-1	Vascular adhesion molecule-1
ICAM-1	Intercellular adhesion-molecule-1
VLA-4	Very late antigen-4
LFA-1	Lymphocyte function-associated antigen-1

There are different types of the clinical course of MS, including the relapsing-remitting MS (RRMS) characterized by single attacks/relapses which can be recovered fully or partially, present at the 80–85% of the MS patients [5,13]. During secondary progressive MS (SPMS), there is an initial RRMS disease course, leading to the serial worsening with or without occasional relapses. Whereas primary progressive MS (PPMS) is characterized by progressive accumulation of disability since disease onset and occurs in around 15–20% of MS patients [13,14]. The most frequently observed MS symptoms include sensory disruptions in the limbs, dysfunction of the optic nerve, dysregulation of pyramidal tract and bladder/bowel, ataxia and diplopia [15].

Food and drug administration (FDA) agency has approved around 12 medications (Alemtuzumab, Ocrelizumab, Mitoxantrone, Natalizumab, Fingolimod, Peginterferon Beta-1a, Dimethyl fumarate, Teriflunomide, Glatiramer acetate, IFNβ-1a, IFNβ-1b, Teriflunomide and IFN β-1a) to arrest the course of MS [16,17]. However, therapeutic agents targeting acute MS exacerbations are still limited (Sun et al. , 2018)., Disease-modifying treatment (DMT) options are limited for both PPMS and SPMS [18] often due to their huge cost [19]. Hence, exploring molecular biomarkers and improvement of DMT strategies against MS are urgently needed. In this regard, high mobility group box-1 (HMGB1) protein has emerged as a potential candidate due to its implication in EAE/MS pathogenesis.

HMGB1 is a pro-inflammatory cytokine-like molecule initiating inflammatory responses mainly by activating toll-like receptor 4 (TLR4) and receptor for the advanced glycation end product (RAGE) [20]. HMGB1 protein is composed of a chain of 215 amino acids with a molecular mass of 25-kDa consisting of two DNA-binding domains, boxes A and B, and a highly conserved negatively charged C-terminal tail composed of aspartic and glutamic acids [21]. In addition to the nuclear expression of HMGB1, it is also released into the extracellular space as a result of cell damage and active secretion. The active secretion of HMGB1 has been detected in several cell types including monocytes, macrophages, dendritic cells (DCs), hepatocytes, glial cells and neurons [22]. HMGB1 exists in three isoforms namely, fully reduced HMGB1, disulfide HMGB1 and sulphonyl HMGB1 [23]. Disulfide HMGB1 is the only one exerting cytokine-inducing activity. The cytokine stimulating activity of HMGB1 depends on the oxidation states of cysteine 106 residing in the B box DNA-binding domain of HMGB1, a region which is crucial for stimulating cytokine release and inflammation [24].

Earlier works has shed lights on the pathogenic role of HMGB1 in an array of neurological diseases including Alzheimer's disease (AD) [25], Huntington's disease (HD) [26], Parkinson's disease (PD) [27,28], and epilepsy [29,30]. Increased levels of HMGB1 in human and experimental MS, strengthen its role in the disease pathogenesis mainly by amplifying inflammatory processes and aggravating neuroinflammation in demyelination [31]. Moreover, HMGB1-based agents have demonstrated a promising outcome mainly through HMGB1 inhibition in the experimental model of MS, namely the experimental autoimmune encephalomyelitis (EAE) [32]; Uzawa et al. , 2013). Herein, we provide a concise review of the mechanistic role of HMGB1 in EAE/MS pathogenesis, further discussing the available HMGB1-targeted therapeutic options for MS and future directions.

2. HMGB1 in the pathogenesis of EAE/MS: evidence from experimental and human MS studies

HMGB1 manipulates immune function by promoting maturation of DCs activation and proliferation of T cells as well as by contributing to the functional polarization of T cells into the pro-inflammatory T helper 1 (Th1) phenotype [33]. HMGB1-mediated microglial activation demonstrates a crucial role in myelin injury as well as in neuronal cell death [34]. Moreover, HMGB1 binding to TLR induces pro-inflammatory cytokine release including tumour necrosis factor-α (TNF-α), interleukin (IL)-1, IL-6 and IL-8 by activated macrophages through Nuclear Factor-κ light chain enhancer of activated B cells (NF-κB) activation [35]. This function of HMGB1 is pathogenically relevant for autoimmune disorders including MS. Emerging evidence has demonstrated the contribution of HMGB1 signaling in MS pathogenesis [36] with the underlying molecular mechanism being still under elucidation.

EAE presents the most widely used experimental model of MS that recapitulates its phenotypes. EAE is characterized by the increased inflammatory activation of CNS glial cells contributing to EAE pathology at all stages of the disease [37]. EAE model can be used to dissect the disease stages and study the processes and mechanisms mediated by astrocytes and glia in neuroimmune disease, perfectly replicating MS pathophysiology [38]. EAE is a $CD4^+$ T cell-driven autoimmune disease exhibiting perivascular inflammation of $CD4^+$ T and mononuclear cells as well as a gradual primary axonal demyelination in the CNS, resulting in progressive hind-limb paralysis. EAE provides a robust model to investigate the pathogenesis and immune regulation of $CD4^+$ T_H1/T_H17-driven tissue damage (Miller et al. , 2010). Brain tissue from EAE demonstrated mononuclear cell infiltration, hemorrhage, vascular congestion, plaques, as along with neuron degeneration [39].

EAE induced by the administration of myelin oligodendrocyte glycoprotein (MOG) protein (200 μg) exhibited elevated serum HMGB1 concentration at all disease stages (pre-onset, onset, peak, and remission) and gradually decreased CSF levels. In spinal cord homogenate, HMGB1 levels peaked only during the onset stage and declined gradually (Sun et al. , 2015). Moreover, the nuclear HMGB1 expression pattern in the astrocytes and microglia remained unchanged during

EAE progression. Elevated expression of HMGB1 was further observed in nuclei of glial fibrillary acidic protein (GFAP) (astrocyte) positive cells in the normal dorsal column (spinal cord). Along with HMGB1, GFAP double-positive cells were subsequently elevated at the peak stage of EAE. Moreover, HMGB1 expression was reported in a few CD11b (microglia) positive cells located in the lateral column (spinal cord). However, the number of $HMGB1^+CD11b^+$ cells was elevated along with the progression of EAE [40]. This finding provides the proof-of-concept that assessment of extracellular HMGB1 levels might be helpful in evaluating the progression and outcome of MS/EAE.

HMGB1 further contributes to neuroinflammatory responses that drive EAE pathogenesis. In a similar experimental MS model, chronic (C)-EAE mice exhibited elevated serum HMGB1 levels compared to control ($OVA_{323-339}$/CFA-primed) but not among mice with different scores. This finding indicates that serum HMGB1 levels increase with the onset of C-EAE however, without reflecting disease scores throughout disease progression (Robinson et al. , 2013). Recombinant HMGB1 (dose-dependently) promoted the proliferation of lymphocyte in response to the myelin epitope without initiating apoptosis. Pro-inflammatory T_H1 (IFN-γ and TNF-α) and T_H17 cytokines (IL-17 and GM-CSF) were observed in the presence of recombinant HMGB1 in a dose-dependent manner. However, recombinant HMGB1 demonstrated no effect on the production of T_H2 cytokine IL-10 suggesting that HMGB1 signaling selectively promotes T_H1/T_H17 cell function [41]. Plasma levels of HMGB1, IL-17, IL-1β, IL-6, IL-10, IL-17A, IFN-γ and TNF-α were upregulated during EAE indicating its possible contribution to the pathogenesis of EAE (Uzawa et al. , 2018). Strong HMGB1 staining has been mainly reported in macrophages ($ED1^+$) and microglia ($Iba1^+$) cells from EAE animals (Djedović et al. , 2017). Similarly, HMGB1 expressed significantly during the progression of EAE, with $HMGB1^+GFAP^+$ (astrocytes) and $HMGB1^+Iba1^+$(microglia) cells been increased significantly in the EAE. Nuclear to cytoplasmic translocation of HMGB1 was observed in the central canal neurons of the spinal cord of EAE [32]. HMGB1 was released from the cell nucleus during brain inflammation and loss of nuclear HMGB1 immunoreactivity during EAE indicated the nuclear HMGB1-induced inflammation [42].

Anderson et al. for the very first time reported significant expression of HMGB1 and its receptor (TLR4, TLR2, and RAGE) in active lesions of human and experimental MS implicating that HMGB1 and its receptors might initiate inflammatory responses to promote the neuroinflammatory processes [31]. They further report that acute MS and RRMS exhibited a high number of macrophages/microglia with cytoplasmic HMGB1 expression whereas lesions from PPMS and SPMS had comparable inactive MS lesions. Moreover, at the immune-mediated model of MS (EAE) [43], HMGB1 signal was not only localized in the multiple lesions but was widespread throughout the spinal cord section, implicating abundant and significant inflammatory response in animals with severe EAE [31]. In addition, high expression of the HMGB1 receptors (RAGE, TLR2, and TLR4) was evident in the active EAE lesions indicating their importance in propagating inflammatory response. This pioneering investigation shed light on the plausible role of HMGB1 in MS and several investigations were further conducted on the ground of this finding. Elevated HMGB1 mRNA expression levels in peripheral blood mononuclear cells (PBMC) were detected in MS patients as compared to healthy controls. However, on stratification of MS patients based on clinical forms, significantly higher HMGB1 mRNA and protein levels were observed in RRMS patients as compared to healthy controls and PPMS patients [44]. Elevated CSF levels of HMGB1, IL-6, and IL-17 observed in MS patients in comparison to healthy controls reflects the induction of the neuroinflammatory process [45] A cross-sectional study further reported elevated serum levels of HMGB1 in MS patients as compared to healthy control, suggesting a possible role of HMGB1 in MS pathogenesis [46]. A recent meta-analysis has presented the striking relationship between HMGB1 levels in MS patients since the increased HMGB1 levels (CSF, serum, and PBMC) were found to correlate with the increased risk for MS [47]. This is attributed to the possibility that HMGB1 may be secreted from activated macrophages, microglia and mature DCs in the CNS during infection, inflammatory stimuli, as well as be released from dead cells during injury. In addition, inflammatory demyelination and neuronal necrosis may actively contribute to MS pathogenesis, thereby initiating HMGB1 secretion and release, leading to upregulated HMGB1 concentration in CSF, serum, and PBMC of MS patients as compared to healthy controls [47].

This evidence strongly associates HMGB1 with the pathogenesis of MS/EAE. Hence, potential beneficial therapeutic effects might be achieved by targeting HMGB1 since they can attenuate CNS inflammation and demyelination, attenuate the progression of EAE, suppress the activation of astrocytes and microglia, reduce infiltrates, attenuate the subsequent release and expression of HMGB1 as well as decrease the expression of HMGB1 in astrocytes and microglia.

3. HMGB1 neutralization as a promising approach against MS/EAE

Due to the unknown pathogenesis of MS, therapeutic evaluation of several HMGB1 targeting therapies including anti-HMGB1 monoclonal antibodies (mAb), glycyrrhizin (pharmacological inhibitor of HMGB1) and other miscellaneous agents (compounds which inhibit HMGB1) against MS/EAE have been instrumental in elucidating the plausible role of HMGB1 in the pathogenesis of MS/EAE.

3.1. HMGB1inhibition reduces the progression of EAE

The clinical progression of the disease is based on a 5 point scale score with slight modification existing between studies, where score 0, no symptoms; score 0.5, abnormal gait or tail tone loss; score 1.0, complete tail paralysis/tail tone loss with abnormal gait; score 1.5, complete tail paralysis and mild hind limb weakness; score 2.0, tail paralysis with moderate hind limb weakness; score 2.5, no weight-bearing on hind limbs some movement; score 3, complete hind limb paralysis; score 3.5, forelimbs weakness; 4.0 complete hind limb and forelimb paralysis; and 4.5 moribund state [48–51]. Treatment with HMGB1 targeting agents has resulted in the attenuation of disease progression and exerted a protective effect against clinical symptoms of EAE. Anti-HMGB1 mAb treatment (5–20 μg) after 11–15 days of MOG administration attenuated the EAE progression as evidenced by a reduction in the clinical score (2.67 ± 0.49 versus 3.17 ± 0.40 for anti-HMGB1 mAb treated and EAE group respectively) [42]. Anti-HMGB1 mAb administration (each alternate day from days 12–22 post-EAE induction) by intraperitoneal injection (I.P.) (100 μg) attenuated the clinical progression of the disease whereas intracerebroventricular (I.C.V.)(10 μg) administration almost completely inhibited the EAE progression, reduced the disease incidence and delayed EAE onset time [40]. Precisely, for the I.P. administration group, the mean maximal score was decreased (2.79 ± 0.20 versus 1.38 ± 0.31) and disease onset score was increased (14.17 ± 0.67 versus 17.33 ± 1.99) for EAE and treated group respectively. In addition, for I.C.V. administration group, mean maximal score was decreased (2.25 ± 0.31 versus 0.63 ± 0.25) and disease onset score was increased (13.38 ± 0.89 versus 14.80 ± 0.37) for EAE and treated group respectively [40]. These findings strengthen the understanding that local HMGB1 in CNS contributes to the EAE progression.

There are different types of EAE with a difference in the course of the disease. For example, in the C57BL/6 mice, the disease exhibited a chronic-progressive clinical course [52], whereas in other strains such as PL/J or B10.PL mouse, the disease is often acute, self-limiting, and devoid of clinical relapses [53]. In addition, in the SJL mice, the disease is characterized by a relapsing-remitting (R-EAE) course of paralysis, reflecting the clinical phenotype of MS in human [54,55]. In a C-EAE and R-EAE model , anti-HMGB1 mAb (100 μg) was administered prophylactically (7 or 14 days post-immunization) and was shown to significantly inhibit the C-EAE disease induction as well as to significantly

ameliorate disease progression In addition, anti-HMGB1 mAb improved clinical R-EAE disease induction when compared to control-treated animals, and prevented the first relapse. However, it did not block future relapses. This finding indicates that HMGB1 is a critical mediator of C-EAE and R-EAE but not the sole factor contributing the EAE pathogenesis [41]. Glycyrrhizin (10, 25, and 50 mg/kg) treatment (on -1 to11 day, 12 to 22 days, or 15 to 23 days after EAE induction) ameliorated the disease severity and exerted long-term protective effects by decreasing the disease incidence as well as by delaying the disease onset. This observation is at least due to its inhibitory effects on HMGB1 expression and to the release of neuronal HMGB1 during EAE [32]. Treatment with 18β-glycyrrhetinic acid (100 mg/kg/day) after 14 days of EAE immunization improved gradually the clinical score, day 9 (0.95 ± 0.09 versus 0.106 ± 0.07), day 17 (3.58 ± 0.09 versus 3.18 ± 0.10) and after 24 days (3.06 ± 0.12 versus 2.08 ± 0.10) [39].

3.2. HMGB1 blockade inhibited CNS inflammation and demyelination in EAE

Administration of neutralizing anti-HMGB1 mAb (5–20 μg, after 11–15 days of MOG administration) reduced cell infiltration as well as demyelination in the spinal cord of EAE mice alone with the inflammatory index. Moreover, anti-HMGB1 mAb treatment ameliorated inflammation, as evidenced by the residual nuclear HMGB1 staining in anti-HMGB1 mAb treated EAE mice spinal cord sections. In addition, IL-17 a contributor to MS pathogenesis was also found reduced upon anti-HMGB1 mAb treatment [42]. Anti-HMGB1 mAb further blocked infiltration of T cell of the CNS and blocked systemic $CD4^+$T cell responses to myelin epitopes. In addition, lymphocytes from EAE mice restimulated *in vitro* in the presence of recombinant HMGB1 demonstrated increased proliferation and production of the pro-inflammatory cytokine, which was inhibited by an anti-HMGB1 antibody. Moreover, recombinant HMGB1 induced proliferation and production of pro-inflammatory cytokine of human PBMC stimulated *in vitro* which was further blocked by anti-HMGB1 antibody [41]. Glycyrrhizin treatment in EAE model reduced CNS demyelination and inflammation, decreased the infiltration of $CD3^+$ T lymphocytes and ameliorated the pathological scores (infiltration and demyelination score). Moreover, treatment with glycyrrhizin downregulated TNF-α, IFN-γ, IL-17A, IL-6, and transforming growth factor (TGF)-β1, and upregulated IL-4 (in serum and spinal cord) suggesting that it may prevent neuroinflammation in EAE [32]. Treatment with 18β-glycyrrhetinic acid (a hydrolysed metabolite of glycyrrhizin) significantly reduced inflammation caused due to EAE as evidenced by decreased TNF-α and IL-1β levels. In addition, it also reduced adverse histopathological changes (mononuclear cell infiltration, necrosis/neuronal loss and neuron degeneration) as well as reduced the number of cells that stained positive for caspase 3 and IL-17 in brain tissue of mice [39].

3.3. HMGB1 neutralization suppresses the activation of astrocytes and microglia in EAE

Microglial cell activation and astrocytes proliferation contribute to MS progression [56,57]. Astrocytes participate as players of the innate immune system and as a source of cytotoxic factors, blocking remyelination and axonal regeneration via formation of a glial scar, and resulting to the dysfunction of axonal mitochondria [58]. Microglial and astrocytes activation and proliferation that are evident within demyelinating lesions signify that innate immune response contribution by CNS cells might possess a key role in oligodendrocyte injury as well as in axonal degeneration [59]. Astrocytes express diverse pattern recognition receptors (PRRs) and may drive innate immune responses [60]. They affect the entry of cell to the CNS via BBB, by monitoring expression of adhesion molecules, especially vascular adhesion molecule-1 (VCAM-1) and intercellular adhesion-molecule-1 (ICAM-1) which bind to lymphocyte receptors, very late antigen-4 (VLA4) and lymphocyte function-associated antigen-1 (LFA-1), respectively [61,62]. Additionally, IL-6, IL-1β, TNF-α, and TGF-β released by astrocytes can monitor the passage of immune cells via BBB, by acting on

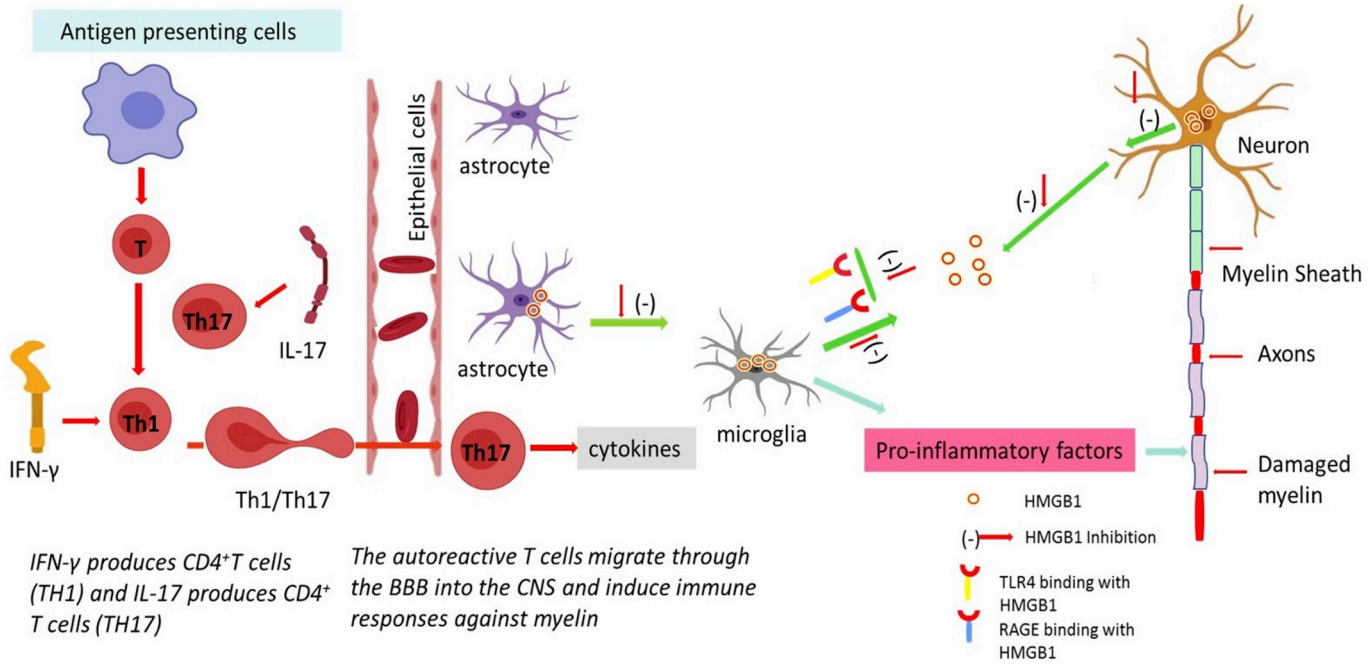

Fig. 1. HMGB1 neutralization strategy against EAE/MS.
Activated peripheral immune cells enter into the CNS by disrupting BBB where microglia and astrocytes are activated and cytokines are released resulting in the neuronal disruption and demyelination [80]. HMGB1 is released from astrocytes, microglia, and neurons and contributes to EAE pathogenesis, which can be blocked by HMGB1 neutralization.
CNS, central nervous system; BBB, blood-brain barrier; EAE, experimental autoimmune encephalomyelitis; HMGB1, high mobility group box protein 1.

endothelial cells and tight junctions [63–65].

Notably, exploring innovative therapeutic strategies targeting astrocytes may exert a possible therapeutic effect against EAE/MS [66]. When the astrocytes and microglia were activated in EAE, the number of GFAP + and Iba1$^+$ cells were upregulated in the total sections (WM) and grey matter (GM) of the spinal cord. Glycyrrhizin treatment decreased the GFAP$^+$ and Iba1$^+$ cells numbers and recovered the number of NeuN$^+$ neurons (reflecting neuronal damage). This finding indicates that glycyrrhizin alleviated EAE by inhibiting both gliosis and microglial cell activation as well as by exerting protection against neuronal damage in the spinal cord [32]. R-EAE mice injected with anti-HMGB1 mAb (100 μg) at the primary remission demonstrated significantly less CD45$^+$ hematopoietic cell infiltration in the ventral spinal cord as compared to control-treated mice. Mice injected with anti-HMGB1 mAb at disease remission significantly downregulated HMGB1 receptors expression (TLR2 and TLR4). Infiltrating immune cells further exhibited low CD3$^+$ and CD4$^+$ T cell numbers in the CNS of anti-HMGB1 treated mice as compared to controls [41].

3.4. HMGB1 neutralization attenuates release and expression of HMGB1 and blocks the translocation of HMGB1 during EAE

It is well-established that at the site of inflammation and/or damage, there is a nuclear to cytoplasmic translocation of HMGB1 via passive release or through active secretion, where it acts as a damage-associated molecular pattern (DAMP) [67]. Treatment with glycyrrhizin was found to significantly inhibit the upregulation of HMGB1 in EAE mice (serum, CSF and spinal cord homogenate). Immunohistochemical staining detected a downregulation of HMGB1 levels (lateral and dorsal column, ventral and dorsal horn) after treatment with glycyrrhizin which was previously upregulated in the EAE group indicating its beneficial effects in reducing the release and expression of HMGB1. Moreover, glycyrrhizin treatment inhibited the translocation of HMGB1 in neurons and it was further suggested to also block the TNF-α-mediated HMGB1 release from neuronal cells in EAE [32].

4. Discussion and Translational implications

MS is characterized by demyelination, axonal loss, activation of glial cells, and infiltration of immune cells (macrophages and lymphocytes) [68]. Continuous understanding of the underlying RRMS disease mechanisms has led to the development of several DMTs, that aim to reduce both the severity and frequency of new relapses, by modulating or suppressing the innate immune system [69]. On the contrary, therapeutic options for progressive MS are disappointing and remain challenging which might be attributed to the lack of understanding of the pathogenic mechanisms mediating progressive MS [58].

It is well known that the immune system plays a crucial role in tissue destruction which is one of the characteristic features of MS, however, the role of specific immune mediators in MS pathogenesis is not fully understood [70]. Therapeutic strategies against autoimmune diseases by targeting the intrinsic DAMPs that drive the local inflammatory response are currently being explored [71–73]. Interestingly, DAMPs exhibit special features of targeting the innate immunity without disrupting the adaptive immune response to pathogens. HMGB1 being a prototypical DAMP has been implicated in MS pathogenesis as evidenced by its high levels in human and experimental MS models followed by the elevated expression of its main principal receptors (RAGE, TLR2, TLR4). Hence, HMGB1 neutralization strategy may prove a promising approach against MS/EAE (Fig. 1). Further exploring the therapeutic potential of HMGB1, neutralizing agents against an experimental model of MS have demonstrated disease-modifying effects plausibly by attenuating the EAE progression, decreasing inflammation and demyelination, blocking activation of microglia and astrocytes and inhibiting HMGB1 release and translocation. The therapeutic effect of HMGB1 neutralizing agents is possibly attributed to its inhibitory effects on HMGB1 expression as well as to the neuronal HMGB1 release during EAE. Despite the compelling association of HMGB1 levels and MS, no studies have reported the clinical outcomes of HMGB1 neutralizing agents in MS patients.

Although HMGB1 neutralizing therapy presents a promising approach against MS/EAE, there are certainly several concerns associated with its efficacy. Firstly, there is only a limited number of studies evaluating HMGB1 targeting agents in EAE as well as in MS patients. However, studies with other therapeutic agents (other than anti-HMGB1 mAb, glycyrrhizin) has shown positive outcomes against EAE and MS patients mainly through HMGB1 inhibition [74–76]. Moreover, pharmacological inhibition of HMGB1 in an inappropriate time might block the process of tissue repair rather than minimize inflammation, reflecting an inherent risk of using anti-HMGB1 treatment in the CNS [31]. HMGB1 is a complex protein that exists in three isoforms (fully reduced HMGB1, disulphide HMGB1 and sulfonyl HMGB1) and disulphide HMGB1 is the only form that has been detected to contribute to inflammation. This reflects the need for developing isoform-specific therapeutic approaches which will overcome the limitations of current HMGB1-based therapy that targets all three isoforms [77]. Moreover, the deeper understanding of the complex biology of HMGB1, its release mechanisms during physiological and disease conditions, will provide valuable information in developing HMGB1 neutralizing agents against MS/EAE targeting extracellular HMGB1 at the right time. Alarmingly, the contribution of HMGB1, mainly CNS-derived HMGB1, in the MS/EAE pathogenesis is not yet fully understood [32]. All the HMGB1 neutralizing agents (anti-HMGB1 mAb, glycyrrhizin) have demonstrated their therapeutic potential only in the experimental model of EAE. Notably, findings are emerging stating that active and passive EAE models might not be an ideal animal model to investigate immunological mechanisms based on disease initiation and relapse [38]. This limitation can be overcome through the use of transgenic models which develop a spontaneous form of EAE [78,79]. Additionally, direct evidence of the exact source and target cells of HMGB1 in MS is highly needed. Exploring the diverse roles of HMGB1 in different stages of the disease will likely advance the therapy of MS and the effort to explore and investigate several HMGB1 inhibitors, have the potential to impact the future of MS therapeutics [80]. Despite these limitations, pre-clinical evidence regarding the therapeutic benefits of targeting HMGB1 in EAE/MS are rather encouraging and indicate that HMGB1 protein may be a potential biomarker and a promising candidate target worth to explore.

Author's contribution

YNP conceived, carried out the literature review and drafted the manuscript. EA, BKC, IO and CP contributed to redrafting the manuscript and provided critical revisions and contributed to the final manuscript. All authors read and approved the final manuscript.

Funding

This work does not receive any sort of financial assistance from any funding agency.

Declaration of competing interest

All the author declares no potential conflict of interest.

References

[1] R. McCool, K. Wilson, M. Arber, K. Fleetwood, S. Toupin, H. Thom, et al., Systematic review and network meta-analysis comparing ocrelizumab with other treatments for relapsing multiple sclerosis, Multiple sclerosis and related disorders 29 (2019) 55–61.

[2] B. Ferguson, M.K. Matyszak, M.M. Esiri, V.H. Perry, Axonal damage in acute multiple sclerosis lesions, Brain: J. Neurol. 120 (1997) 393–399.

[3] B.G. Weinshenker, Epidemiology of multiple sclerosis, Neurol. Clin. 14 (1996) 291–308.
[4] B.G. Weinshenker, B. Bass, G. Rice, J. Noseworthy, W. Carriere, J. Baskerville, et al., The natural history of multiple sclerosis: a geographically based study: 2 predictive value of the early clinical course, Brain 112 (1989) 1419–1428.
[5] B.G. Weinshenker, B. Bass, G. Rice, J. Noseworthy, W. Carriere, J. Baskerville, et al., The natural history of multiple sclerosis: a geographically based study: I. Clinical course and disability, Brain 112 (1989) 133–146.
[6] S. Glatigny, E. Bettelli, Experimental autoimmune encephalomyelitis (EAE) as animal models of multiple sclerosis (MS), Cold Spring Harbor Perspect. Med. 8 (2018) a028977.
[7] D.S. Goodin, E. Frohman, G. Garmany, J. Halper, W. Likosky, F. Lublin, Disease modifying therapies in multiple sclerosis, Neurology 58 (2002) 169–178.
[8] L. Calvo-Barreiro, H. Eixarch, X. Montalban, C. Espejo, Combined therapies to treat complex diseases: the role of the gut microbiota in multiple sclerosis, Autoimmun. Rev. 17 (2018) 165–174.
[9] P. Browne, D. Chandraratna, C. Angood, H. Tremlett, C. Baker, B.V. Taylor, et al., Atlas of multiple sclerosis 2013: a growing global problem with widespread inequity, Neurology 83 (2014) 1022–1024.
[10] M. Chao, S. Ramagopalan, B. Herrera, S. Orton, L. Handunnetthi, M. Lincoln, et al., MHC transmission: insights into gender bias in MS susceptibility, Neurology 76 (2011) 242–246.
[11] M. Hashemilar, D. Savadi Ouskui, M. Farhoudi, H. Ayromlou, A. Asadollahi, Multiple sclerosis in East-Azerbaijan, north west Iran, Neurol. Asia 16 (2011) 127–131.
[12] J. Poorolajal, M. Mazdeh, M. Saatchi, E.T. Ghane, A. Biderafsh, B. Lotfi, et al., Multiple sclerosis associated risk factors: a case-control study, Iran. J. Public Health 44 (2015) 1498.
[13] A. Nack, M. Brendel, J. Nedelcu, M. Daerr, S. Nyamoya, C. Beyer, et al., Expression of translocator protein and [18F]-GE180 ligand uptake in multiple sclerosis animal models, Cells 8 (2019) 94.
[14] R. Richards, F. Simpson, S. Beard, P. Tappenden, A Review of the Natural History and Epidemiology of Multiple Sclerosis: Implications for Resource Allocation and Health Economic Models, (2002).
[15] C.R. Smith, L.C. Scheinberg, Clinical features of multiple sclerosis, Seminars in Neurology: © 1985 by Thieme, Medical Publishers, Inc., 1985, pp. 85–93.
[16] C. English, J.J. Aloi, New FDA-approved disease-modifying therapies for multiple sclerosis, Clin. Ther. 37 (2015) 691–715.
[17] H. Li, F. Hu, Y. Zhang, K. Li, Comparative efficacy and acceptability of disease-modifying therapies in patients with relapsing–remitting multiple sclerosis: a systematic review and network meta-analysis, J. Neurol. (2019) 1–10.
[18] J.R. Ciotti, A.H. Cross, Disease-modifying treatment in progressive multiple sclerosis, Curr. Treat. Options Neurol. 20 (2018) 12.
[19] M. Zimmermann, E. Brouwer, J.A. Tice, M. Seidner, A.M. Loos, S. Liu, et al., Disease-modifying therapies for relapsing–remitting and primary progressive multiple sclerosis: a cost-utility analysis, CNS Drugs 32 (2018) 1145–1157.
[20] Y.N. Paudel, E. Angelopoulou, C. Piperi, V.R. Balasubramaniam, I. Othman, M.F. Shaikh, Enlightening the role of high mobility group box 1 (HMGB1) in inflammation: updates on receptor signalling, Eur. J. Pharmacol. (2019) 172487.
[21] U. Andersson, H. Yang, H. Harris, Extracellular HMGB1 as a therapeutic target in inflammatory diseases, Expert Opin. Ther. Targets 22 (2018) 263–277.
[22] U. Andersson, H. Rauvala, Introduction: HMGB1 in inflammation and innate immunity, J. Intern. Med. 270 (2011) 296–300.
[23] H. Aucott, J. Lundberg, H. Salo, L. Klevenvall, P. Damberg, L. Ottosson, et al., Neuroinflammation in response to intracerebral injections of different HMGB1 redox isoforms, J. Innate Immun. 10 (2018) 215–227.
[24] J. Li, R. Kokkola, S. Tabibzadeh, R. Yang, M. Ochani, X. Qiang, et al., Structural basis for the proinflammatory cytokine activity of high mobility group box 1, Mol. Med. 9 (2003) 37–45.
[25] K. Takata, Y. Kitamura, J-i Kakimura, K. Shibagaki, D. Tsuchiya, T. Taniguchi, et al., Role of high mobility group protein-1 (HMG1) in amyloid-β homeostasis, Biochem. Biophys. Res. Commun. 301 (2003) 699–703.
[26] A.-V. Goula, B.R. Berquist, D.M. Wilson III, V.C. Wheeler, Y. Trottier, K. Merienne, Stoichiometry of base excision repair proteins correlates with increased somatic CAG instability in striatum over cerebellum in Huntington's disease transgenic mice, PLoS Genet. 5 (2009) e1000749.
[27] E. Angelopoulou, C. Piperi, A.G. Papavassiliou, High-mobility group box 1 in Parkinson's disease: from pathogenesis to therapeutic approaches, J. Neurochem. 146 (3) (2018 Aug) 211–218.
[28] T. Sasaki, K. Liu, T. Agari, T. Yasuhara, J. Morimoto, M. Okazaki, et al., Anti-high mobility group box 1 antibody exerts neuroprotection in a rat model of Parkinson's disease, Exp. Neurol. 275 (2016) 220–231.
[29] M. Maroso, S. Balosso, T. Ravizza, J. Liu, E. Aronica, A.M. Iyer, et al., Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures, Nat. Med. 16 (2010) 413.
[30] Y.N. Paudel, B.D. Semple, N.C. Jones, I. Othman, M.F. Shaikh, High mobility group box 1 (HMGB 1) as a novel frontier in epileptogenesis: from pathogenesis to therapeutic approaches, J. Neurochem. (2019).
[31] Å. Andersson, R. Covacu, D. Sunnemark, A.I. Danilov, A. Dal Bianco, M. Khademi, et al., Pivotal advance: HMGB1 expression in active lesions of human and experimental multiple sclerosis, J. Leukoc. Biol. 84 (2008) 1248–1255.
[32] Y. Sun, H. Chen, J. Dai, Z. Wan, P. Xiong, Y. Xu, et al., Glycyrrhizin protects mice against experimental autoimmune encephalomyelitis by inhibiting HMGB1 expression and neuronal HMGB1 release, Front. Immunol. 9 (2018) 1518.
[33] I.E. Dumitriu, P. Baruah, B. Valentinis, R.E. Voll, M. Herrmann, P.P. Nawroth, et al., Release of high mobility group box 1 by dendritic cells controls T cell activation via the receptor for advanced glycation end products, J. Immunol. 174 (2005) 7506–7515.
[34] M.L. Block, Neuroinflammation: modulating mighty microglia, Nat. Chem. Biol. 10 (2014) 988.
[35] M. Penzo, R. Molteni, T. Suda, S. Samaniego, A. Raucci, D.M. Habiel, et al., Inhibitor of NF-κB kinases α and β are both essential for high mobility group box 1-mediated chemotaxis, J. Immunol. 184 (2010) 4497–4509.
[36] C. Zhen, Y. Wang, D. Li, W. Zhang, H. Zhang, X. Yu, et al., Relationship of High-mobility group box 1 levels and multiple sclerosis: a systematic review and meta-analysis, Mult. Scler. Relat. Disord. 31 (2019) 87–92.
[37] C.S. Constantinescu, N. Farooqi, K. O'Brien, B. Gran, Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS), Br. J. Pharmacol. 164 (2011) 1079–1106.
[38] R. Brambilla, The contribution of astrocytes to the neuroinflammatory response in multiple sclerosis and experimental autoimmune encephalomyelitis, Acta Neuropathol. (2019) 1–27.
[39] S. Kamisli, O. Ciftci, A. Taslidere, N. Basak Turkmen, C. Ozcan, The beneficial effects of 18β-glycyrrhetinic acid on the experimental autoimmune encephalomyelitis (EAE) in C57BL/6 mouse model, Immunopharmacol. Immunotoxicol. 40 (2018) 344–352.
[40] Y. Sun, H. Chen, J. Dai, H. Zou, M. Gao, H. Wu, et al., HMGB1 expression patterns during the progression of experimental autoimmune encephalomyelitis, J. Neuroimmunol. 280 (2015) 29–35.
[41] A.P. Robinson, M.W. Caldis, C.T. Harp, G.E. Goings, S.D. Miller, High-mobility group box 1 protein (HMGB1) neutralization ameliorates experimental autoimmune encephalomyelitis, J. Autoimmun. 43 (2013) 32–43.
[42] A. Uzawa, M. Mori, J. Taniguchi, S. Masuda, M. Muto, S. Kuwabara, Anti-high mobility group box 1 monoclonal antibody ameliorates experimental autoimmune encephalomyelitis, Clin. Exp. Immunol. 172 (2013) 37–43.
[43] M.K. Storch, A. Stefferl, U. Brehm, R. Weissert, E. Wallström, M. Kerschensteiner, et al., Autoimmunity to myelin oligodendrocyte glycoprotein in rats mimics the spectrum of multiple sclerosis pathology, Brain Pathol. 8 (1998) 681–694.
[44] S. Malhotra, N. Fissolo, M. Tintoré, A.C. Wing, J. Castilló, A. Vidal-Jordana, et al., Role of high mobility group box protein 1 (HMGB1) in peripheral blood from patients with multiple sclerosis, J. Neuroinflammation 12 (2015) 48.
[45] H. Wang, K. Wang, C. Wang, F. Xu, X. Zhong, W. Qiu, et al., Cerebrospinal fluid high-mobility group box protein 1 in neuromyelitis optica and multiple sclerosis, Neuroimmunomodulation 20 (2013) 113–118.
[46] Z. Sternberg, D. Sternberg, T. Chichelli, A. Drake, N. Patel, C. Kolb, et al., High-mobility group box 1 in multiple sclerosis, Immunol. Res. 64 (2016) 385–391.
[47] C. Zhen, Y. Wang, D. Li, W. Zhang, H. Zhang, X. Yu, et al., Relationship of High-mobility group box 1 levels and multiple sclerosis: a systematic review and meta-analysis, Mult. Scler. Relat. Disord. 31 (2019 Jun 1) 87–92.
[48] A. Cauwels, S. Van Lint, D. Catteeuw, S. Pang, F. Paul, E. Rogge, et al., Targeting interferon activity to dendritic cells enables in vivo tolerization and protection against EAE in mice, J. Autoimmun. 97 (2019) 70–76.
[49] L. Legroux, A.C. Moratalla, C. Laurent, G. Deblois, S. Verstraeten, N. Arbour, NKG2D and its ligand MULT1 contribute to disease progression in a mouse model of multiple sclerosis, Front. Immunol. 10 (2019) 154.
[50] T. Matsushita, K. Yanaba, J.-D. Bouaziz, M. Fujimoto, T.F. Tedder, Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression, J. Clin. Investig. 118 (2008) 3420–3430.
[51] S. Pol, F. Schweser, N. Bertolino, M. Preda, M. Sveinsson, M. Sudyn, et al., Characterization of leptomeningeal inflammation in rodent experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, Exp. Neurol. 314 (2019 Apr 1) 82–90.
[52] A. Farinazzo, S. Angiari, E. Turano, E. Bistaffa, S. Dusi, S. Ruggieri, et al., Nanovesicles from adipose-derived mesenchymal stem cells inhibit T lymphocyte trafficking and ameliorate chronic experimental autoimmune encephalomyelitis, Sci. Rep. 8 (2018) 7473.
[53] S.D. Miller, W.J. Karpus, T.S. Davidson, Experimental autoimmune encephalomyelitis in the mouse, Curr. Protoc. Im. 88 (2010) 15 1. 1-.1. 20.
[54] X. He, X. Qiu, Q. GUO, X. Liu, H. Luo, D. Fan, et al., Pien tze huang alleviates relapsing-remitting experimental autoimmune encephalomyelitis mice by regulating Th1 and Th17 cells, Front. Pharmacol. 9 (2018) 1237.
[55] S. Lunin, M. Khrenov, O. Glushkova, S. Parfenyuk, T. Novoselova, E. Novoselova, Immune response in the relapsing-remitting experimental autoimmune encephalomyelitis in mice: the role of the NF-κB signaling pathway, Cell. Immunol. 336 (2019) 20–27.
[56] E.N. Benveniste, Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis, J. Mol. Med. 75 (1997) 165–173.
[57] A. Williams, G. Piaton, C. Lubetzki, Astrocytes—friends or foes in multiple sclerosis? Glia 55 (2007) 1300–1312.
[58] J. Correale, M.F. Farez, The role of astrocytes in multiple sclerosis progression, Front. Neurol. 6 (2015) 180.
[59] L. Mayo, F.J. Quintana, H.L. Weiner, The innate immune system in demyelinating disease, Immunol. Rev. 248 (2012) 170–187.
[60] C. Farina, F. Aloisi, E. Meinl, Astrocytes are active players in cerebral innate immunity, Trends Immunol. 28 (2007) 138–145.
[61] M.A.T. Gimenez, J.E. Sim, J.H. Russell, TNFR1-dependent VCAM-1 expression by astrocytes exposes the CNS to destructive inflammation, J. Neuroimmunol. 151 (2004) 116–125.
[62] R.A. Sobel, M.E. Mitchell, G. Fondren, Intercellular adhesion molecule-1 (ICAM-1) in cellular immune reactions in the human central nervous system, Am. J. Pathol. 136 (1990) 1309.
[63] N.J. Abbott, L. Rönnbäck, E. Hansson, Astrocyte–endothelial interactions at the

blood–brain barrier, Nat. Rev. Neurosci. 7 (2006) 41.
[64] N. Didier, I.A. Romero, C. Créminon, A. Wijkhuisen, J. Grassi, A. Mabondzo, Secretion of interleukin-1β by astrocytes mediates endothelin-1 and tumour necrosis factor-α effects on human brain microvascular endothelial cell permeability, J. Neurochem. 86 (2003) 246–254.
[65] A. Minagar, J.S. Alexander, Blood-brain barrier disruption in multiple sclerosis, Mult. Scler. J. 9 (2003) 540–549.
[66] H.M. Ta, T.M. Le, H. Ishii, M. Takarada-Iemata, T. Hattori, K. Hashida, et al., Atf6α deficiency suppresses microglial activation and ameliorates pathology of experimental autoimmune encephalomyelitis, J. Neurochem. 139 (2016) 1124–1137.
[67] H.E. Harris, U. Andersson, D.S. Pisetsky, HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease, Nat. Rev. Rheumatol. 8 (2012) 195.
[68] L. Legroux, N. Arbour, Multiple sclerosis and T lymphocytes: an entangled story, J. Neuroimmune Pharmacol. 10 (2015) 528–546.
[69] J.A. Cohen, R.A. Rudick, Multiple Sclerosis Therapeutics, Cambridge University Press, 2011.
[70] C. Baecher-Allan, B.J. Kaskow, H.L. Weiner, Multiple sclerosis: mechanisms and immunotherapy, Neuron 97 (2018) 742–768.
[71] H. Chen, Y. Sun, L. Lai, H. Wu, Y. Xiao, B. Ming, et al., Interleukin-33 is released in spinal cord and suppresses experimental autoimmune encephalomyelitis in mice, Neuroscience 308 (2015) 157–168.
[72] S.-Y. Seong, P. Matzinger, Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses, Nat. Rev. Immunol. 4 (2004) 469.
[73] Y. Xiao, L. Lai, H. Chen, J. Shi, F. Zeng, J. Li, et al., Interleukin-33 deficiency exacerbated experimental autoimmune encephalomyelitis with an influence on immune cells and glia cells, Mol. Immunol. 101 (2018) 550–563.
[74] N. Djedović, S. Stanisavljevic, B. Jevtić, M. Momčilović, I. Lavrnja, D. Miljković, Anti-encephalitogenic effects of ethyl pyruvate are reflected in the central nervous system and the gut, Biomed. Pharmacother. 96 (2017) 78–85.
[75] Z. Sternberg, C. Kolb, K. Chadha, A. Nir, R. Nir, R. George, et al., Fingolimod anti-inflammatory and neuroprotective effects modulation of RAGE axis in multiple sclerosis patients, Neuropharmacology 130 (2018) 71–76.
[76] A. Uzawa, M. Mori, H. Masuda, R. Ohtani, T. Uchida, S. Kuwabara, Recombinant thrombomodulin ameliorates experimental autoimmune encephalomyelitis by suppressing high mobility group box 1 and inflammatory cytokines, Clin. Exp. Immunol. 193 (2018) 47–54.
[77] U. Andersson, H. Yang, H. Harris, High-mobility group box 1 protein (HMGB1) operates as an alarmin outside as well as inside cells, Seminars in Immunology, Elsevier, 2018.
[78] I. Bjelobaba, V. Begovic-Kupresanin, S. Pekovic, I. Lavrnja, Animal models of multiple sclerosis: focus on experimental autoimmune encephalomyelitis, J. Neurosci. Res. 96 (2018) 1021–1042.
[79] J. Goverman, A. Woods, L. Larson, L.P. Weiner, L. Hood, D.M. Zaller, Transgenic mice that express a myelin basic protein-specific T cell receptor develop spontaneous autoimmunity, Cell 72 (1993) 551–560.
[80] J. Li, J. Shi, Y. Sun, F. Zheng, Glycyrrhizin, a potential drug for autoimmune encephalomyelitis by inhibiting high-mobility group box 1, DNA Cell Biol. 37 (2018) 941–946.