https://doi.org/10.5607/en24002
Exp Neurobiol. 2024 Apr;33(2):47-67.
pISSN 1226-2560 • eISSN 2093-8144

Review Article

Experimental Neurobiology

Possible Roles of Extracellular Vesicles in the Pathogenesis and Interventions of Immune-Mediated Central Demyelinating Diseases

Chutithep Teekaput[1], Kitti Thiankhaw[1], Nipon Chattipakorn[2,3] and Siriporn C. Chattipakorn[2,3,4]*

[1]Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, [2]Neurophysiology Unit, Cardiac Electrophysiology Research and Training Center, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, [3]Center of Excellence in Cardiac Electrophysiology, Chiang Mai University, Chiang Mai 50200, [4]Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Chiang Mai 50200, Thailand

Multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) are two of the most devastating immune-mediated central demyelinating disorders. NMOSD was once considered as a variant of MS until the discovery of an antibody specific to the condition. Despite both MS and NMOSD being considered central demyelinating disorders, their pathogenesis and clinical manifestations are distinct, however the exact mechanisms associated with each disease remain unclear. Extracellular vesicles (EVs) are nano-sized vesicles originating in various cells which serve as intercellular communicators. There is a large body of evidence to show the possible roles of EVs in the pathogenesis of several diseases, including the immune-mediated central demyelinating disorders. Various types of EVs are found across disease stages and could potentially be used as a surrogate marker, as well as acting by carrying a cargo of biochemical molecules. The possibility for EVs to be used as a next-generation targeted treatment for the immune-mediated central demyelinating disorders has been investigated. The aim of this review was to comprehensively identify, compile and discuss key findings from *in vitro*, *in vivo* and clinical studies. A summary of all findings shows that: 1) the EV profiles of MS and NMOSD differ from those of healthy individuals, 2) the use of EV markers as liquid biopsy diagnostic tools appears to be promising biomarkers for both MS and NMOSD, and 3) EVs are being studied as a potential targeted therapy for MS and NMOSD. Any controversial findings are also discussed in this review.

Key words: Demyelination, Multiple sclerosis, Neuromyelitis optica spectrum disorders, Extracellular vesicles, Exosome

INTRODUCTION

Multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD), both immune-mediated demyelinating disorders of the central nervous system (CNS), have been acknowledged as being some of the most debilitating neurological conditions. MS is a chronic immune-mediated demyelinating disorder that primarily affects Europeans, and is uncommon in Asians, Africans, and indigenous Americans [1]. It has been suggested that the etiology of MS is centrally demyelination induced as a consequence of inflammatory cells invading the CNS through blood vessels in the blood-brain barrier (BBB) [2]. These inflammatory cells, in addition to activated microglia and astrocytes, have been shown to contribute to oligodendrocyte injury [1, 3, 4]. The relapsing-remitting phenotype of MS, which accounts for 85-90% of all MS cases, is caused by peripheral inflammation as a result of periodic invasion [4, 5]. In the advanced progressive stage of MS, the repetitive destruction of oligodendrocytes causes persistent oxidative stress, mitochondrial dysfunction, brain iron accumulation, hypoxia, defective remyelination, and glutamate hyperexcitability, eventually

Submitted January 30, 2024, Revised April 16, 2024,
Accepted April 28, 2024

*To whom correspondence should be addressed.
TEL: 011-66-53-944-451, FAX: 011-66-53-222-844
e-mail: scchattipakorn@gmail.com

leading to neurodegeneration [1, 6]. The clinical manifestations of MS vary based on the area affected, frequent locations being the optic nerve, the cerebral cortex, white matter, brainstem, and spinal cord [1]. Disease-modifying therapies (DMTs), which primarily target inflammatory cascades and have pleiotropic effects, have been the standard therapies for MS [3]. The exact pathophysiology of MS, however, remains unknown, and no specific antibody has been identified and investigated facilitating the detection of MS. As a result, the diagnosis of MS still utilizes the 2017 McDonald criteria which indicate space and time dissemination [7].

Until 2004 NMOSD was considered an MS variant but then a specific antibody was discovered [8]. That antibody is immunoglobulin G (IgG) directed against aquaporin 4 (AQP4), in combination with complement system activation [8, 9]. That antibody causes invasion of the CNS by peripheral granulocytes through the CNS, resulting in astrocytic endfeet damage, severe inflammation, secondary oligodendrocytic injury, and demyelination [9, 10]. The six essential clinical characteristics that must be present in addition to the presence of AQP4 IgG for a diagnosis of NMOSD are longitudinally extensive transverse myelitis (LETM), optic neuritis (ON), area postrema syndrome (APS), brainstem encephalitis, diencephalitis, and telencephalitis [9]. Autoantibody elimination and inflammatory cascade suppression have been the main goals of NMOSD therapy [9, 11, 12].

Extracellular vesicles (EVs) are nano-sized, non-nucleated structures that are hypothesized to facilitate intercellular communication and contribute to autoimmune processes. EVs have been connected to the development and deterioration of a range of pathological conditions, including cancer, cerebrovascular disease, Alzheimer's disease, and central demyelinating diseases (MS and NMOSD) [13-17]. Many studies have described the potential involvement of EVs as possible biomarkers and as a novel diagnostic approach known as a liquid biopsy for a variety of disorders [18-20]. Furthermore, one of the therapeutic uses of EVs which has the greatest potential is as carrier for the drug delivery system [21]. As a result of those previous studies, the aim of this review was to summarize and discuss the evidence available from *in vitro*, *in vivo*, and clinical studies on the role of EVs in central demyelinating diseases, primarily as regards MS and NMOSD, as well as EV therapy strategies for treatment of central demyelinating diseases. Any contradictory findings have been included and considered in this review.

SEARCH STRATEGY AND SELECTION CRITERIA

The PubMed database was searched using the keywords: "*demyelinating*", "*multiple sclerosis*", "*neuromyelitis optica*", "*exosome*", "*extracellular vesicles*", "*microvesicles*" and "*central nervous system*" with a demarcation from 2011 to 2024. The search was limited to research articles published in the English language.

OVERVIEW OF EXTRACELLULAR VESICLES

EVs are double-layered and non-nucleated vesicles that contain a diversity of cytoplasmic biological substances. Different cell types, in both physiological and pathological stages, secrete distinctive EVs in order to maintain cellular homeostasis and communicate with adjacent cells [22]. Considered as a carrier for biochemical compounds, enzymes, signaling molecules, biogenesis factors, chaperones, cytoskeletal factors, and nucleic acids especially microRNA are contained within EVs. Based on their biogenesis, release mechanisms, size, composition, and function, the three primary subtypes of extracellular vesicles (EVs) are exosomes, microvesicles, and apoptotic bodies [13, 23-26]. Exosomes are the smallest EVs, ranging from 30~100 nm in diameter, and are formed by the inward budding of the endosomal compartment, subsequently being released to the periphery by the exocytotic process. Exosomes usually contain lipids and nucleic acids [24]. Microvesicles, however, are approximately 100~1,000 nm in diameter and since they arise directly from the cell membrane, they typically express transmembrane molecules and glycoproteins depending on their parental cells [25]. Apoptotic bodies are approximately 1,000 to 5,000 nm in diameter and mostly develop in the late stage of apoptotic cells. Phosphatidylserine is often transferred to the outer membrane of apoptotic bodies, which then stimulates phagocytes to remove the dead cells [26]. As previously stated, EVs are involved in cell-to-cell communication; hence, dysfunctional EVs lead to pathogenic processes and promote autoimmunity [27].

ISOLATION AND CHARACTERIZATION OF EXTRACELLULAR VESICLES

Extracted EVs from MS and NMOSD patients were investigated using various methods. However, the interpretation and comparison of the findings across studies must be considered carefully. Different isolation and characterization techniques used in the different studies caused different EV findings [28]. Focusing on the isolation technique, ultracentrifugation (UC) is the most widely used isolation method and is considered the gold standard for EV extraction and separation. UC does not need EV labeling and can avoid cross-contamination. However, this technique is time-consuming and expensive [22]. Polymer precipitation is another approach that uses polyethylene glycol as a medium, the technique involves the incubation of EVs under centrifugation conditions

https://doi.org/10.5607/en24002

by decreasing the EV solubility. Contrarily, this approach has a high false-positive rate, and the polymer has an adhesive feature that makes it difficult to remove for future EV investigation. Size-based isolation by means of size exclusion chromatography is an alternative technology with a high enrichment efficiency and low cost. The main disadvantage of this technique is its impurity [22, 28]. The different characterization techniques also can have impact on different EV characteristics. Characterization techniques are mainly divided into external characterization (primarily morphology and particle size detection) and internal characterization (membrane protein and lipid raft). The electron microscope is used to detect EV morphology, but the technique is complicated and unsuitable for the rapid testing of many EV samples. Nanoparticle tracking analysis (NTA) is a real-time detection method but carries a high risk of impurity [28]. Camera levels and detection thresholds affect the quality of NTA. Western blotting is a conventional technique that can qualitatively and quantitatively analyze marker proteins. However, the detection of marker proteins varies depending on the type of parental cell, and it is not suitable for the detection of EV marker proteins in biological fluids [28].

Since the quantity of EVs in blood plasma or serum is believed to be between 10^7 and 10^{12} EV/ml, serum EVs have garnered a great deal of attention [29]. However, many investigators have demonstrated the EVs in various samples beyond serum. Different sample types can result in different EV profiles. In contrast with serum, cerebrospinal fluid (CSF) is a type of biological fluid secreted from choroid plexus ependymal cells. CSF surrounds the brain and spinal cord in order to protect the CNS, support homeostasis, and remove waste products. Approximately 80% of CSF EVs originate from the blood, whereas the remaining are produced by nerve cells [30]. However, due to the high integrity of the BBB, the protein level of CSF becomes 100 to 300 times lower than protein found in serum [30, 31]. Therefore, a high amount of CSF is required in order to obtain sufficient EVs for analysis. Albumin and immunoglobulin, the two most abundant proteins in the CSF, may hinder the identification of low-abundance proteins and result in a substantial loss of EV information. As a result, direct comparisons between methods may be difficult according to the advantages and disadvantages of individual techniques. Size, function, sample type, and parental source of EV make isolating and characterization of EV problematic [28, 32].

EXTRACELLULAR VESICLES AND PATHOLOGICAL CORRELATIONS IN CENTRAL DEMYELINATING DISEASES

Extracellular vesicles and pathological correlations in multiple sclerosis

The central paradigm of central demyelinating diseases, especially in the case of MS, is an increase in the inflammatory process and a downregulation of immune tolerance. EVs derived from various origins carry different characteristics and functions.

Extracellular vesicles classified by their cellular origins

Endothelial cell derived extracellular vesicles

The pathophysiology of MS is thought to involve endothelial dysfunction as a crucial factor. The expression of certain markers on the endothelial surface, such as CD31 (PECAM-1), CD51 (integrin), CD54 (ICAM-1), CD62E (ELAM-1 or E-selectin), CD63E, and CD105 (endoglin), increases the adhesion of inflammatory leukocytes to endothelial cells and promotes their transendothelial migration into the CNS [33-37].

It is believed that radiological exacerbation, which refers to an increase in the severity of lesions seen on imaging without the presentation of any clinical symptoms, occurs prior to or simultaneously with clinical exacerbation. Notably, CD31 and CD54, which are localized at endothelial cell intercellular junctions, show high levels of expression during radiological relapse even in the absence of clinical symptoms [34, 36]. Once the exacerbation reaches the clinical exacerbation threshold, endothelial cell damage occurs, leading to the disruption of the BBB. CD31 and CD62E are among the earliest markers to be released, indicating endothelial injury. CD51, along with CD54, which acts as a transmembrane receptor, facilitates leukocyte recruitment and adhesion to endothelial cells via Th1 cell activation [34, 36, 38]. CD51 also plays a role in signaling between the extracellular matrix and cells and has been found to indirectly contribute to nerve regeneration. Following an exacerbation, demyelinated neurons attempt to repair and promote remyelination. The expression of CD51 increases after an attack but remains elevated even after treatment, suggesting that remyelination is a long-term process [39]. However, not all brain regions exhibit the same levels of these endothelial EV markers. Based on the process of neurovascular injury, the subcortical gray matter periventricular region, which is highly vascularized, is the most affected area of the brain [35]. The association between different brain regions may provide additional insights from imaging data for predicting the clinical course and early diagnosis of relapse.

Platelet-derived extracellular vesicles

Platelets have been classified as inflammatory cells due to their

multifaceted functions beyond hemostasis. Within platelet-alpha granules reside a wide range of EVs responsible for inflammatory process [40]. Following BBB disruption from impaired endothelial cells and astrocyte foot processes, peripheral inflammatory cells are recruited into the CNS. Platelet-derived EVs have been proposed to play a significant role in leukocyte recruitment and to facilitate transendothelial migration into the CNS, leading to inflammation and demyelination. In patients with MS, CD31+ marker of platelet EV is observed only in the inflammation-predominant stage, RRMS [33, 35]. This phenomenon is not evident in the very early stage, CIS, nor in the advanced stage, SPMS. The postulated explanation is that there is limited degree of inflammation demonstrated in CIS, whereas in SPMS, neurodegeneration rather than neuroinflammation is the primary pathophysiology [41]. However, some level of inflammation persists across all stages of MS evidenced by the heightened expression of CD61 (integrin beta 3), CD62 (P-selectin), and surface IgM on platelets [33, 42, 43]. The alteration of platelet-derived EVs could potentially serve as a marker for identifying MS stages and predicting MS prognosis [33, 35].

Leukocyte-derived extracellular vesicles

Inflammation arises from an imbalance between the increase in proinflammatory leukocytes and the decrease of regulatory T cells (Treg). A recent study has found that specific markers of T cells in CSF, such as CCR3 and CCR5, exhibit high expression during radiological relapse, but not during clinical relapse [44]. It is suggested that activated CD4+ and CD8+ T lymphocytes play a significant role in triggering inflammation. An in vitro study has demonstrated that co-culturing human endothelial cells with T cell EVs leads to a decrease in transendothelial electrical resistance, indicating BBB disruption and astrocyte damage [45, 46]. Furthermore, both in vitro and clinical studies have shown that B cells impact the reduced function of oligodendrocytes [47, 48]. In conjunction with Treg dysfunction, the apoptotic rate of inflammatory leukocytes decreases, resulting in the proliferation of the inflammatory leukocyte population. The excessive accumulation of leukocytes subsequently leads to inflammatory damage within the CNS. However, both T cells and B cells predominantly contribute to the inflammatory stage seen in RRMS. In SPMS, there is a significant decrease in CD14 and CD45 expression, suggesting that neurodegeneration can surpass the extent of neuroinflammation [49].

Microglia/macrophage-derived extracellular vesicles

Microglia, which are considered to be resident macrophages in the CNS, play a crucial role in maintaining self-tolerance and preventing autoimmunity. It has been hypothesized that once leukocytes enter the CNS compartment, microglia may contribute to the promotion of inflammation. In patients with MS, microglial EVs have been found to be significantly upregulated, even in the CIS [50]. During exacerbations, markers associated with microglia/macrophages, such as IB4, showed a significant increase, indicating inflammation mediated by microglia [51]. In an in vitro model, EVs derived from activated microglia/macrophages were shown to impair synaptic function, as evidenced by reduced expression of markers like Syt1 and N1g1 [52]. Additionally, microglia/macrophage EVs might hinder remyelination by inhibiting the accumulation of oligodendrocyte progenitor cells and reducing levels of myelin basic protein (MBP) [53].

Oligodendrocyte-derived extracellular vesicles

Following demyelination, oligodendrocytes play a role in promoting remyelination. Myelin oligodendrocyte glycoprotein (MOG) is secreted by oligodendrocytes in response to demyelination. An elevated level of MOG expression is indicative of increased inflammation. The available evidence suggests that MOG expression in the remitting stage of RRMS is similar to that in healthy individuals but becomes highly expressed during relapsing and progressive stages [54]. Another protein, MBP, also serves as a marker of demyelination. A significant increase in MBP levels in the serum is observed across all stages, whereas healthy individuals do not exhibit such elevation [55]. These findings suggest a potential association between EVs released by oligodendrocytes and the demyelinating response.

Astrocyte-derived extracellular vesicles

Although astrocytes are the primary target of AQP4-IgG in NMOSD, it has also been observed that EVs derived from astrocytes are present in the experimental autoimmune encephalomyelitis (EAE) model of MS. The EVs obtained from EAE mice showed a significantly higher level of glial fibrillary acidic protein (GFAP). Since GFAP is essential for maintaining the cytoskeleton of astrocytes, the increased expression of GFAP in CSF suggests the presence of astrocyte damage and inflammation [56]. However, it is important to note that astrocyte dysfunction may not be the primary mechanism underlying the pathogenesis of MS, even though it is widely accepted as a central process in NMOSD. The subsequent section will provide a more detailed of NMOSD.

Extracellular vesicles categorized by surface membrane expression

Extracellular vesicles with fibrinogen-laden membranes

Fibrinogen-laden EVs are recognized for their significant in-

https://doi.org/10.5607/en24002

volvement in inflammation, extending beyond their traditional role in coagulation processes [57]. Within the CN, various neuroglial cells, including oligodendrocytes, astrocytes, and microglia, express receptors for fibrinogen, facilitating their interaction with fibrinogen-laden EVs and subsequently leading to the activation of encephalitogenic CD8+ T cell responses. In the EAE model, mice injected with fibrinogen-enriched EVs demonstrated the development of encephalitogenic T cell responses, while this phenomenon was not observed in the fibrinogen-deficient group. Laboratory confirmation of inflammatory markers such as CXCL10 and CCL2 provided evidence supporting the proposed involvement of the fibrinogen alpha chain in stimulating CD8+ T cells [58]. Furthermore, fibrinogen-laden EVs were observed to directly transfer antigens and pathogenic peptides to antigen-presenting cells (APCs) via major histocompatibility complex (MHC) class I complexes on their surface membrane [59]. In summary, it is hypothesized that fibrinogen-laden EVs may contribute to human leukocyte antigen (HLA) class I disequilibrium and susceptibility to autoimmunity, ultimately playing a role in the development of MS.

Extracellular vesicles with glycolipid membranes

Sulfatide belongs to the sulfoglycolipid group and is associated with myelin formation. Beginning in the endoplasmic reticulum and finishing up in the Golgi apparatus, ceramide is transformed to galactocerebroside which then is sulfated to produce sulfatide. In addition to being a membrane constituent, sulfatide is also associated with protein transportation, intercellular communication, and glial-axon interactions [60]. Recently, a study found that sulfatide mediated immunomodulatory mechanisms in certain conditions such as autoimmunity, infections, and cancers [61]. In MS, a specific sulfatide fraction known as C16:0 exhibited distinct differences compared to those without MS and showed inverse correlation with the expanded disability status scale (EDSS) [62]. These pathways of immune regulation are primarily triggered by the activation of type II natural killer T (NKT) cells which identify sulfatides via CD1d molecules [63]. C16:0 sulfatide exerts an anti-inflammatory role by inhibiting cytokine production. Additionally, the over-functioning of the acid sphingomyelinase enzyme (ASMase), which is usually seen in MS patients, enhances the hydrolysis of other healthy sphingomyelin into ceramide, resulting in a reduction of sphingomyelin and subsequent inflammation [64]. Changes in the quantities, distribution, and metabolism of glycolipids ultimately exacerbate MS.

Extracellular vesicles as cargo of biological substances

Protein, peptides, and enzymes

At least 50 CSF proteins showed significant increases compared to healthy individuals [65]. ASMase, responsible for sphingolipid metabolism, was highly expressed in CSF. ASMase is also associated with ASMase-dependent ceramide signaling, which coordinates intracellular signal cascades and governs the activation and proliferation of CD4+ T-cells. ASMase can also induce neuronal mitochondrial dysfunction and axonal damage [64]. Another protein, Apolipoprotein E4 (ApoE4), was found to be enriched in the CSF of MS individuals [65]. ApoE4 is associated with neurodegeneration due to decreased clearance of pathogenic amyloid-β, as commonly seen in many neurological diseases, especially Alzheimer's disease [66]. This finding may explain the subsequent neurodegeneration observed in SPMS. Other proteins such as KLKB1, DKK3, C6, and S100A9 were also analyzed and found to be responsible for scar healing, homeostasis, and clot formation, as commonly observed as sequelae of demyelination [65]. However, limited data were gathered due to the very small amount of EV extracted from the CSF. Additionally, CSF is not routinely taken in clinical practice.

MicroRNAs

MicroRNA consists of an 18 to 22-nucleotide single-stranded non-coding molecule. MicroRNA genes are generally transcribed by RNA polymerase II within the nucleus and are subsequently processed in the cytoplasm. The mature microRNA is finally transported into the extracellular space in order to control cell-to-cell communication. Circulating microRNAs have been found to have a correlation with various conditions and diseases, even in the case of MS. Many studies have demonstrated the role of microRNA as an inflammatory trigger in serum, and it also confers a degree of immune protection. A study found suppressed function of anti-inflammatory T cells called Foxhead box P3 (FOXP3+) Treg is observed in MS patients. Let-7i is markedly increased in the serum of MS patients and suppresses the function of insulin-like growth factor 1 receptor (IGF1R) and transforming growth factor beta receptor 1 (TGFβR1). IGF1 and TGFβR1 are important for Treg differentiation and clonal expansion, and impairment of both functions subsequently promotes inflammation. Increments of miR-15b-5p, miR-30b-5p, miR-342-3p, and miR-451a were observed in RRMS patients [67]. MiR-15b and miR-23a were found to be targeted at fibroblast growth factor 2 (FGF-2), which is linked to inflammation, demyelination, and remyelination. MiR-23a was also found to be involved with the level of oligodendrocyte differentiation. A higher level of miR-23a meant there was substantial oligodendrocyte damage. MiR-30b and miR-342 had previously

been recognized as potential biomarkers for Alzheimer's disease and Parkinson's disease [68-71]. The additional association with MS pathology of both miRNAs apparently supported their contribution to neuro-axonal damage in CNS [72]. In contrast, miR-122-5p, miR-196b-5p, miR-301a-3p, and miR-532-5p, which are considered immune protection EVs, are downregulated in MS patients [72]. MiR-122-5p inhibits inflammatory cell proliferation and migration. MiR-196b-5p is secreted from hematopoietic cells in both normal conditions and malignancy. MiR-301a-3p is a critical endogenous regulator of Th17 cell proliferation and maturation. MiR-532-5p can suppress the inflammatory pathways and has previously been reported as a tumor suppressor in ovarian cancer [72].

Not only serum EVs, but also CSF-derived EVs have been investigated in central demyelinating diseases. Upregulation of miR-18a-5p, miR-21, miR-30a-5p, miR-145-5p, miR-150, miR-328, miR-342-3p, miR-374a-5p, miR-645, and Let-7g-5p, together with the down-regulation of miR-106a, miR-132-5p, miR-146a, miR-191, miR-199a-3p, miR-320a-5p, and miR-365 were associated with oligoclonal band production in CSF and inflammation [73, 74]. Increased miR-181c and miR-633 with a reduction in miR-922, were all associated with inflammatory suppression and may be associated with the reactive response to inflammation [75]. Even though there are several studies on MS and miRNAs in the CSF, they do not directly claim the miRNAs found in these studies were EV-associated. Different extraction techniques, study design, and validation procedures contributed to the variability of results in EV studies [76-79].

Several studies have tried to demonstrate the differences of EVs in serum and CSF to explain the exact pathogenesis of MS [54, 80, 81]. Major myelin proteins, including MPB, proteolipid protein, and MOG, were substantially elevated in the serum and CSF in MS patients. However, peripheral blood mononuclear cell (PBMC)-derived EVs, considered as a potential peripheral EV source, failed to cause expression of MOG or any other myelin proteins. In addition, evidence showed that myelin-associated EVs could transmigrate from the CNS into the periphery through the BBB. This result suggested that MS pathology may originate in the CNS and then spread to the periphery [56]. Another study focusing on miRNAs in MS patients found that serum and CSF miRNAs differed from healthy controls and varied across different disease stages. At least twenty-one miRNAs were significantly expressed, mainly upregulated during an MS relapse, in comparison with the clinical remission stage. However, the level of some miRNAs, such as miR-21-5p, miR-142-3p, miR-223-3p, miR-342-3p, miR-423-5p, and let-7f-5p, were also highly expressed in other CNS inflammatory and infectious diseases [82]. The expression of miRNA was associated with pleocytosis and the existence of oligoclonal bands, which were not only unique to MS but also associated with intrathecally immune-mediated processes such as CNS infection and inflammation. However, due to a lack of specificity, using a single miRNA alteration as a surrogate marker for MS may not be applicable, and a combination of miRNA panels may be suitable for this purpose. All data from clinical studies are shown in Table 1 and 2. Table 3 and Table 4 show the data from in vivo and in vitro studies, respectively.

Extracellular vesicles and pathological correlations in neuromyelitis optica spectrum disorder (NMOSD)

Given the underlying issue of astrocyte dysfunction in NMOSD, individuals with this condition typically show increased expression of astrocyte-associated markers. A recent study has revealed that EVs obtained from CSF, such as GFAP, tetraspanin, haptoglobin-related protein, and C4b binding protein-alpha, were significantly upregulated in NMOSD but not in MS [81]. This discovery suggests that the mechanisms underlying BBB dysfunction differ between MS and NMOSD. Experimental models and human NMOSD lesions have demonstrated a transient disruption of the BBB caused by infiltration of immune cells including macrophages and polymorphonuclear cells (PMNs) with only a few T and B cells, a phenomenon absent in MS [83]. The migration of PMNs into the brain could additionally exacerbate NMOSD lesion formation by allowing additional AQP-4 antibodies and complement to enter the brain. The activation of the complement cascade during astrocyte destruction, in turn, results in the release of the anaphylatoxin C5a, a potent PMN chemoattractant and activator leading to a vicious inflammatory cycle [84].

Alteration of circulating miRNAs may serve as possible biomarkers for NMOSD. A study demonstrated miRNA dysregulation, as shown by an increase in miRNA-122-3p and miR-200a-5p, which occurs in NMOSD, but not in MS [85]. The miR-122-3p targets the MAPK signaling pathway associated with both innate and adaptive autoimmunity [86]. The miR-200a-5p targets the Ras and Wnt signaling pathways, modulating cell proliferation and the inflammatory response. Additionally, the elevation of miR-122-3p is correlated with the increasing EDSS, indicating that miR-122-3p might be a potential biomarker for the prediction of NMOSD relapse. Comparable to other well-defined autoimmune diseases, EV alteration in systemic lupus erythematosus (SLE) is associated with the inflammatory pathway and disease progression. Serum EV miR-21 and miR-155 of SLE patients were upregulated, whereas the expression of miR-146a was down-regulated. Even though the specific mechanism of EVs is unclear, these EVs might activate inflammatory cells via the TLR7 pathway, allowing them

https://doi.org/10.5607/en24002

Table 1. Extracellular vesicle marker in central demyelinating diseases related to inflammatory pathways from plasma or serum: reports from *clinical studies*

Methods of EV detection (specimen)	Major findings of each disorder								Interpretation	Ref
	Multiple sclerosis	Subtypes of multiple sclerosis				NMOSD	Relapsing disease activity			
		RRMS	SPMS	PPMS	CIS		Clinical	Radiologic		
EVs classified by origin										
Endothelial cells										
Flow cytometry (platelet-poor plasma)	↑ PMP ↑ CD31 ↑ CD62E	↑ PMP ↑ CD31 ↔ CD62E	↑ PMP ↑ CD31 ↔ CD62E	↑ PMP ↑ CD31 ↔ CD62E	↔ PMP ↑ CD31 ↑ CD62E	NA	NA	NA	An increase in EMPs-CD31 involved in all courses of MS. However, PMP was associated with chronic MS but not with early stage, increased EMP-CD62E was found in the early stage of disease.	[33]
Flow cytometry and 3T MRI (plasma)	NA	↑ CD31+/ CD51+ / CD61+/CD54+ (EMPs)	↓CD31+/ CD51-/CD61-/ CD54- (PMPs)	NA	NA	NA	NA	NA	An increase in EMPs was more common in RRMS than in SPMS. PMP levels rose in SPMS but not in SPMS.	[35]
Flow cytometry, scanning EM (platelet-poor plasma)	↑ CD105	NA	NA	NA	NA	NA	NA	NA	Untreated MS had high endothelial microparticles detected from CD105.	[93]
Flow cytometry (platelet-poor plasma)	NA	↑ CD51+	NA	NA	NA	NA	↑ CD31+ ↑ CD51+	↑ CD31+	CD31+ associated with clinical and radiologic exacerbation of MS, while CD51+ was associated with natural course of MS.	[34]
Flow cytometry with fluorescent dye (plasma)	NA	NA	NA	NA	NA	NA	↑ CD54+-monocyte conjugate ↑ CD63E+	↑ CD54+-monocyte conjugate	EMP triggered monocyte-CD54+ binding and activation.	[36]
Flow cytometry (plasma)	NA	NA	NA	NA	NA	NA	↑ CD31 ↑ CD54 ↑ CD62E	NA	EMP associated with exacerbation of MS.	[90]
Tandem spectrometry (plasma)	↑ Fibrinogen alpha-chain	↑ Fibrinogen alpha-chain	NA	NA	NA	NA	NA	NA	Fibrinogen EV associated with CD8+ activation and caused RRMS.	[58]
Platelet										
Flow cytometry (platelet-rich plasma)	↑ CD62p ↑ PMP ↔ Platelet surface IgG ↑ Platelet surface IgM	NA	NA	NA	NA	NA	NA	NA	MS patients had a high level of long-platelet EV activation.	[43]
Flow cytometry (plasma)	↑ CD61 ↔ CD45 ↔ CD14	↑ CD61 ↑ CD45 ↑ CD14	↔ CD61 ↔ CD45 ↔ CD14	NA	NA	NA	NA	NA	Platelet EV, but not leukocyte EV, played a role in early MS while SPMS reduced all PMP and LMP due to stage changes in neurodegeneration.	[42]
Leukocytes and microglia										
Flow cytometry (plasma)	↑ CD61 ↔ CD45 ↔ CD14	↑ CD61 ↑ CD45 ↑ CD14	↔ CD61 ↔ CD45 ↔ CD14	NA	NA	NA	NA	NA	Platelet EV, but not leukocyte EV, played a role in early MS while SPMS reduced all PMP and LMP due to stage changes in neurodegeneration.	[42]
Immunoprecipitation, NTA	↑ AMPB (T-cell EVs) ↑ FIBB (T-cell EVs) ↑ GELS (B-cell EVs)	NA	NA	NA	NA	NA	↑ GELS (B-cell EVs)	NA	Both T and B cell-dervied EVs correlated with MS. Only B cell associated with active disease.	[48]
Oligodendrocytes										
ExoQuick,NTA, Western blot, ELISA (serum)	NA	↔ MOG	↑ MOG	NA	NA	NA	↑ MOG	↑ MOG	Progressive disease activity correlated with MOG level.	[54]
Exo-check, immuno-gold TEM	NA	↑ Myelin basic protein	NA	↑ Myelin basic protein	↑ Myelin basic protein	NA	NA	NA	Increased oligodencrocyte-dervied EVs associated with MS in all stages.	[55]
EVs categorized by EV surface membrane expression										
Fibrinogen										
Tandem spectrometry (plasma)	↑ Fibrinogen alpha-chain	↑ Fibrinogen alpha-chain	NA	NA	NA	NA	NA	NA	Fibrinogen EV associated with CD8+ activation and caused RRMS.	[58]
Glycolipids										
UC, EM, NTA, Negative ion electrospray mass spectrometry (plasma)	↑ C16:0 sulfatide	↑ C16:0 sulfatide	↑ C16:0 sulfatide	NA	NA	NA	NA	NA	Sulfatide C16:0 triggered CD1d and activated T cell.	[62]

Table 1. Continued

Methods of EV detection (specimen)	Major findings of each disorder									Ref
	Multiple sclerosis	Subtypes of multiple sclerosis				NMOSD	Relapsing disease activity		Interpretation	
		RRMS	SPMS	PPMS	CIS		Clinical	Radiologic		
EVs classified by containing substances										
microRNAs										
High throughput NGS (serum)	NA	↑ miR-15b-5p ↑ miR-30b-5p ↑ miR-342-3p ↑ miR-451a	↑ miR-127-3p ↑ miR-370-3p ↑ miR-409-3p ↑ miR-432-5p ↑ miR-15b-5p ↑ miR-223-3p ↑ miR-23-3p	↑ miR-127-3p ↑ miR-370-3p ↑ miR-409-3p ↑ miR-432-5p ↑ miR-15b-5p ↑ miR-223-3p ↑ miR-23-3p	NA	NA	↓ miR-30b-5p ↓ miR-342-3p ↓ miR-374a-5p ↑ miR-432-5p ↑ miR-433-3p ↑ miR-485-3p	NA	Dysregulated miRNA associated with MS subtypes.	[67]
NGS (serum)	NA	↓ miR-122-5p ↓ miR-196b-5p ↓ miR-301a-3p ↓ miR-532-5p	NA	NA	NA	NA	↓ miR-122-5p ↓ miR-196b-5p ↓ miR-301a-3p ↓ miR-532-5p	↓ miR-122-5p ↓ miR-196b-5p ↓ miR-301a-3p ↓ miR-532-5p	Decreased miRNA was associated with the exacerbation of disease	[72]
High throughput NGS (serum)	NA	NA	NA	NA	↑ miR-126-5p ↑ let-7f-5p ↑ let-7a-5p ↑ miR-23a-3p ↑ miR-223-3p ↓ let-7b-5p ↓ miR-24-3p ↓ let-79-5p ↓ miR-25-3p	NA	NA	NA	CIS patients had different patterns of serum exosomes when compared with HC.	[77]
miScript miRNA techniques RT-PCR (serum)	NA	NA	↑ miR-376c-3p ↑ miR-191-5p ↑ miR-26a-5p	↑ miR-376c-3p ↑ miR-191-5p ↑ miR-26a-5p ↑ miR-128-3p ↑ miR-24-3p	NA	NA	NA	NA	miR-128-3p and miR-24-3p were associated with PPMS.	[78]
NGS with RT-qPCR. EV detection by CD63, CD81 (serum)	↔ miR-380-3p ↔ miR-216a-5p ↔ miR-548p ↔ miR-153-3p ↔ miR-448	↔ miR-380-3p ↔ miR-216a-5p ↔ miR-548p ↔ miR-153-3p ↔ miR-448	NA	NA	NA	↑ miR-122-3p ↓ miR-4424 ↓ miR-6764-3p ↓ miR-412-3p ↓ miR-380-3p ↓ miR-216a-5p ↓ miR-548p ↓ miR-153-3p ↓ miR-448	↑ miR-200a-5p	NA	Mir-122-3p was unique in NMOSD and miR-200a-5p correlated with clinical NMOSD relapse.	[85]
Microarray, Flow cytometry (peripheral blood)	↑ Let-7i	NA	NA	NA	NA	NA	NA	NA	Let-7i inhibited Treg differentiation by inhibiting IGF1R and TGFBR1.	[79]

CIS, clinically isolated syndrome; EMP, endothelial microparticles; MS, multiple sclerosis; EM, electron microscope; EV, extracellular vesicles; HC, healthy controls; ICAM1, Intercellular Adhesion Molecule 1; IGF1R, insulin-like growth factor 1; LMP, leukocyte microparticles; MHC, major histocompatibility complex; miRNA, microribonucleic acid; MOG, myelin oligodendrocyte glycoprotein; NGS, next-generation sequencing; NMOSD, neuromyelitis optica spectrum disorder; NTA, nanoparticle tracking analysis; PECAM, Platelet endothelial cell adhesion molecule; PMP, platelet microparticles; PPMS; primary progressive multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; RT-PCR, reverse transcription polymerase chain reaction; RT-qPCR, quantitative reverse transcription polymerase chain reaction; SPMS, secondary progressive multiple sclerosis; TEM, transmission electron microscope; TGFBR1, Transforming Growth Factor Beta Receptor 1; UC, ultracentrifugation, WM, white matter.

to constantly release IFN-α and proinflammatory cytokines, which may contribute to the pathogenesis of SLE. Additionally, one study reported that the alteration of the Ras signaling pathway enhanced inflammatory T cell function and triggered autoimmunity [87]. Hence, the overlap of Ras signaling pathway dysfunction across two diseases suggests that EVs may promote self-intolerance and autoimmunity.

Contrarily, certain microRNAs probably play a protective effect on NMOSD patients. An in vitro study demonstrated that co-culturing miR-129-2-3p with oligodendrocytes and rat optic nerve resulted in a reduction of demyelination [88]. These results affirm the dual role of microRNAs in NMOSD, where they can act both as anti-inflammatory and pro-inflammatory agents.

In the antibody-negative NMOSD group the majority had evidence of myelin oligodendrocyte glycoprotein antibody-associated disorders (MOGAD). However, until now, there has been no published data that demonstrates any association between EVs and MOGAD. Further studies are still required. The roles of EVs in MS and NMOSD were summarized in Fig. 1.

CURRENT THERAPEUTIC STRATEGIES AND EXTRACELLULAR VESICLE ALTERATION

Since the launch of an investigation into the first disease-modifying therapy (DMT) as a therapeutic target for central demyelinating diseases. Recent studies have shown an association between DMTs and the alterations in EV profiles. Focusing on Interferon-β or IFNβ (including IFNβ-1a and IFNβ-1b), IFNβ could lessen the expression of endothelial surface EVs. The increases in platelet and leukocyte EVs confirmed the reduction of transendothelial migration from the periphery into the CNS [42, 89-91]. Fingolimod inhibited S1P receptor activity, causing lymphocyte sequestration in peripheral lymph nodes and the prevention of lymphocyte migration into the CNS. An increase in platelet EVs, leukocyte EVs, and monocyte EVs, was demonstrated in the plasma of fingolimod-treated individuals confirming that fingolimod can

https://doi.org/10.5607/en24002

Table 2. Extracellular vesicle markers in central demyelinating diseases related to inflammatory pathways from csf: reports from *clinical studies*

Methods of EV detection	Major findings of each disorder								Interpretation	Ref
	Multiple sclerosis	Subtypes of multiple sclerosis				NMOSD	Relapsing disease activity			
		RRMS	SPMS	PPMS	CIS		Clinical	Radiologic		
EVs classified by origin										
Astrocyte										
Proteosome analysis, western blot	↔ GFAP ↔ Tetraspanin ↔ Haptoglobin related protein ↔ C4b binding protein alpha ↑ fibronectin	NA	NA	NA	NA	↑ GFAP ↑ Tetraspanin ↑ Haptoglobin related protein ↑ C4b binding protein alpha ↔ Fibronectin	NA	NA	Exosomes in NMOSD differed from MS. Exosomes played role in BBB dysfunction, inflammatory induction, and astrocyte damage. Exosomes in MS only showed impaired myelination markers but not astrocyte damage.	[81]
Lymphocytes and microglia										
Flow cytometry with Nanoparticle tracking Analysis	NA	↔ IB4	↔ IB4	↔ IB4	↔ IB4	NA	↓ IB4 ↓ $CD19^{+}$/$CD200^{+}$	↑ CCR3 ↑ CCR5 ↑ CD4/CCR3 ↑ CD4/CCR5 ↑ CCR3/CCR5	Clinical relapse was associated with a decrease in naive B cell and microglia EVs. In radiologic relapse, the markers CD4+ and CD8+ T cells were significantly elevated.	[44]
Slow off-rate modified DNA aptamer assay with cluster grouping analysis	NA	↑ MMP7 ↑ SERPINA3 ↑ GZMA ↑ CLIC1 ↑ DSG2 ↑ TNFRSF25 ↑ CXCL13	↑ MMP7 ↑ SERPINA3 ↑ GZMA ↑ CLIC1 ↓ SPARC ↑ DSG2 ↑ TNFRSF25	↑ MMP7 ↑ SERPINA3 ↑ GZMA ↑ CLIC1 ↑ DSG2 ↑ TNFRSF25 ↑ CXCL13	NA	NA	NA	NA	Microglia and myeloid cell EVs were seen in all phases of MS. The expression of toxic astrocyte-specific markers rises with the duration of MS.	[50]
Flow cytometry, RT-PCR	NA	↔ IB4	NA	↔ IB4	↑ IB4	NA	↑ IB4	NA	Resident microglia EVs were abundant in CIS, also association with in clinical recurrence.	[51]
Oligodendrocytes										
ExoQuick,NTA, Western blot, ELISA	NA	↑ MOG	↑ MOG	NA	NA	NA	↑ MOG	NA	MOG correlated with MS.	[54]
EVs classified by containing substances										
Proteins and enzymes										
Flow cytometry, Nanoparticle tracking, immunoblotting	NA	↑ 50 types of CSF protein	NA	NA	NA	NA	NA	NA	At least 50 proteins increased in CSF of RRMS patients.	[65]
Liquid chromatography, western blot, flow cytometry	↑ ASMase	NA	NA	NA	NA	NA	NA	NA	CSF exosomes of MS contained higher ASMase levels.	[64]
microRNA										
TEM, western blotting, RT-PCR	NA	↑ Let-7 g-5p ↑ miR-18a-5p ↑ miR-145-5p ↑ miR-374a-5p ↑ miR-150-5p ↑ miR-342-3p ↓ miR-132-5p ↓ miR-320a-5p	NA	NA	NA	NA	NA	NA	Higher anti-inflammatory EV and lower pro-inflammatory associated with MS in order to prevent further demyelination.	[74]

ASMase, acid sphingomyelinase; CCR3, C-C Motif Chemokine Receptor 3; CCR5, C-C Motif Chemokine Receptor 5; CIS, clinically isolated syndrome. CLIC1, Chloride Intracellular Channel 1; CSF, cerebrospinal fluid; cxcl13, C-X-C Motif Chemokine Ligand 13; DSG2, Desmoglein 2; ELISA, enzyme-linked immunosorbent assay; EV, extracellular vesicles; FITC, Fluorescein isothiocyanate; GFAP, Glial fibrillary acidic protein; GZMA, Granzyme A; HC, healthy controls; IB4, isolectin-B4; MMP7, matrix metalloproteinase 7; MOG, myelin oligodendrocyte glycoprotein; MP, microparticles; MS, multiple sclerosis; NMOSD, neuromyelitis optica spectrum disorder; NTA, nanoparticle tracking analysis; PPMS, primary progressive multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; RT-PCR, reverse transcription polymerase chain reaction; SERPINA3, Serpin Family A Member 3; SPMS, secondary progressive multiple sclerosis; TEM, transmission electron microscope; TNFRSF25, TNF Receptor Superfamily Member 25.

Table 3. Targeting extracellular vesicle markers in central demyelinating diseases related to inflammatory pathways: data from *in vivo* studies

Mechanisms	Model and Methods	Multiple sclerosis	NMOSD	Interpretation	Ref
Fibrinogen activation	EAE mice with MOG33-35 injection. EV analysis by flow cytometry	↑ Cxcl10 ↑ Ccl2	NA	Fibrinogen EV triggered CD8+ function and caused MS.	[58]
Myeloid activation	EAE mice with Th1/LPS-primed microglia EV injection	↑ GFAP ↑ CD86 ↑ iNOS ↑ IL6 ↑ IL1β ↑ COX ↑ CD45 ↑ Iba1+ ↓ CD206	NA	Microglia EVs were found to be responsible for generating inflammation and suppressing remyelination.	[51]
Astrocytes	EAE mice with exosome isolation and detected with flow cytometry	↑ GFAP	NA	Astrocyte-derived exosome associated with demyelination.	[56]

ASMase, acid sphingomyelinase; Ccl2, Chemokine (C-C motif) ligands 2; CIS, clinically isolated syndrome; COX, cyclooxygenase; Cxcl10, C-X-C Motif Chemokine Ligand 10; EAE, experimental autoimmune encephalitis; EV, extracellular vesicle; GFAP, Glial fibrillary acidic protein; iNOS, inducible nitric oxide synthase; MOG, myelin oligodendrocyte glycoprotein; MP, microparticles; NA, not available; NMOSD, neuromyelitis optica spectrum disorder; PPMS, primary progressive multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis.

Table 4. Targeting extracellular vesicle markers in central demyelinating diseases related to inflammatory processes: data from *in vitro* studies

Mechanisms	Model and Methods	Multiple sclerosis	NMOSD	Interpretation	Ref
Treg cell	Mononuclear cells cultured with Treg extract exosome	↑ T cell proliferation ↓ Apoptotic rate	NA	Dysregulated Treg induced T cell proliferation and decreased T cell apoptosis causing inflammation.	[49]
T cell	Human cerebral microvascular endothelial cell + CCL4/CCL5 with TEER measurement	↓ TEER change	NA	CCL4 and CCL5 enhanced BBB dysfunction.	[45]
	Astrocytes co-cultured with MS patient-derived T cells	↑ IFN-γ ↑ IL-17A ↑ CCL2	NA	EVs from astrocytes can stimulate T cells into pro-inflammatory stage.	[46]
B cell	Oligodendrocyte from rat brain cultured in exosome enrich extracts	↑ Cell surface components ↑ External cell membrane components	NA	B cells are the key instigators of the pathogenesis of MS. Key cargo of B cell exosomes in MS derived from cell surface and external cell membrane components.	[47]
Endothelial cells	Endothelial microvesicle detection from cultured human brain microvascular endothelial cell	↑ CD4+ ↑ CD8+	NA	EMP stimulated CD4+ and C8+ T cell proliferation.	[37]
	Human cerebral microvascular endothelial cell + TNFα and measure transendothelial migration assay	↑ Monocyte transmigration	NA	Endothelial EV enhanced adhesion of monocyte to endothelium and migration of monocyte	[36]
Activated microglia	Astrocyte cultured with microglia	↓ Syt1 ↓ N1g1	NA	Activated microglia derived exosomes associated with synaptic dysfunction.	[52]
Platelet and endothelial cells	TEER measurement	↑ F-actin ↑ VE-adherin ↑ ZO-1	NA	Platelet and endothelial derived EV triggered BBB dysfunction in MS.	[33]
Synthetic EV	Microglia cultured with OPC and IL4	↑ Argenase 1 ↑ MBP ↑ OPC accumulation	NA	Pro-regenerative exposed microglia-induced remyelination.	[53]
	Microglia cultured with OPC and Th1 inflammatory cytokines	↑ IL-1a ↑ C1q ↑ IL-1β ↑ iNOS ↓ MBP	NA	Pro-inflammatory exposed microglia inhibited remyelination.	
	Astrocyte cultured with OPC and microglia derived Th1 inflammatory cytokines	↓ MBP ↓ OPC accumulation	NA	Astrocyte exposed microglia-derived EV and inflammatory cytokines inhibited OPC.	
	Adeno-associated virus (AAV) with miR-129-2-3p cultured with oligodencrocyte and rat optic nerve tissue	NA	↓ *Demyelination*	miR-129-2-3p targeting SMAD3 gene can inhibit demyelinating process in NMOSD	[88]

BBB, blood brain barrier; CCL4, Chemokine (C-C motif) ligands 4; CCL5, Chemokine (C-C motif) ligands 5; CIS, clinically isolated syndrome. EMP, Endothelial microparticles; EV, extracellular vesicles; IL, interleukin; iNOS, inducible nitric oxide synthase; MBP, myelin basic protein; MS, multiple sclerosis; NA, not available; NMOSD, neuromyelitis optica spectrum disorder; OPC, oligodendrocyte progenitor cells; PBMC, peripheral blood mononuclear cells; PPMS, primary progressive multiple sclerosis; RRMS, relapsing-remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; TEER, transendothelial electrical resistance; Th1, T helper 1 cell; TNFα, tumor necrosis factor alpha; Treg, regulatory T cells.

hinder immune cells from peripheral transmigration into the CNS [92]. Fingolimod also modified the expression of EVs as shown by a decrease in the expression of endothelium-derived EVs such as CD105, which inhibited the adhesion of inflammatory cells to the BBB [93]. Natalizumab, a humanized monoclonal antibody, modified platelets and lymphocyte EVs, resulting in immune cell suppression and 4-integrin adhesion on activated endothelial surfaces [92]. DMTs which can limit the peripheral inflammatory cells, are preferable treatments since most inflammatory cells originate from outside the CNS rather than within it. Considering that DMTs were responsible for changes in EV levels, the measurement of blood EVs may be useful surrogate-markers for the assessment of therapeutic efficacy in MS. However, more studies are required to add weight to these findings and assess this possibility further. All of these findings are summarized in Table 5.

In contrast, the mainstay treatment of NMOSD is immunosuppressants. In acute exacerbation, intravenous methylprednisolone or plasma exchange is the current major therapy [9]. In the maintenance phase, immunosuppressive drugs including azathioprine, mycophenolate mofetil, methotrexate and rituximab are prescribed to NMOSD patients [9]. However, the data on NMOSD treatment and EVs is scant. Further study in the field is still required.

EXTRACELLULAR VESICLES AS THE POTENTIAL TREATMENT STRATEGIES IN CENTRAL DEMYELINATING DISEASES

Currently, Investigators have been focusing on the role of EVs as a drug delivery tool. EVs are naturally produced and seldom induce intolerance, therefore they have been considered as next-generation possible therapeutic strategies. Mesenchymal EVs are one of the possible groups of EVs being explored in therapeutic approaches for MS [94-96]. Several researchers have hypothesized that placental mesenchymal cells could have a protective function in MS, since previous studies showed decreased exacerbating rates of MS in pregnant women, especially in the third trimester, and a rapid emergence in exacerbation rates after delivery [97]. At various points throughout pregnancy, the maternal circulation becomes flushed with a wide range of EVs representing the maternal-fetal interaction. Pregnant women secrete numerous EVs to suppress the immune response caused by the presence of fetal antigens. Approximately 20% of circulating exosomes in the pregnant woman are of placental origin, and their concentration increases during pregnancy, reaching their peak in the third trimester. Placental EVs originated from various kinds of cells. In a normal physiologic state, after the blastocyte adheres to the placenta, fetal trophoblastic cells differentiate into cytotrophoblasts

https://doi.org/10.5607/en24002

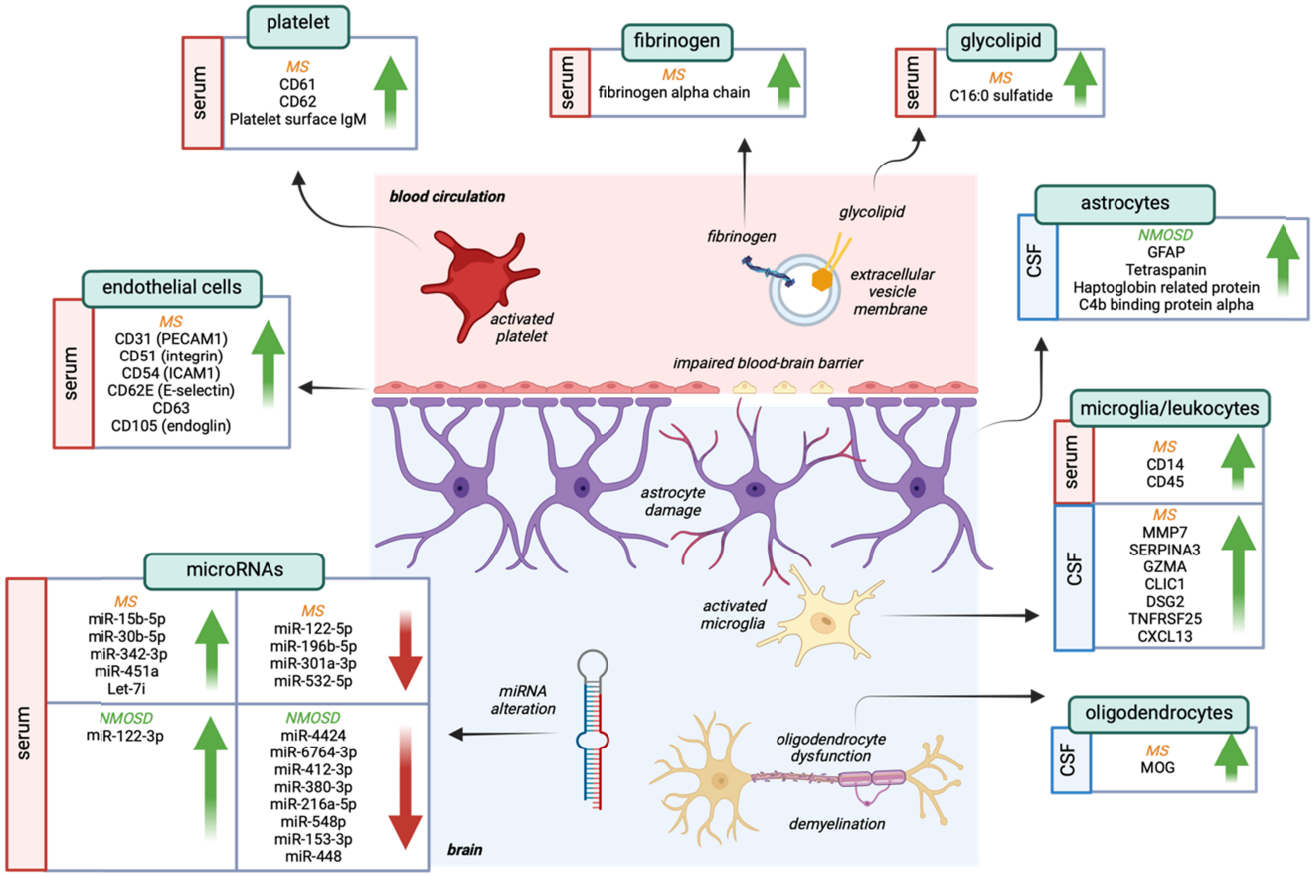

Fig. 1. Extracellular vesicles from serum and cerebrospinal fluid in immune-mediated central demyelinating diseases. Multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) are designated as central demyelinating diseases. Blood-brain barrier disruption caused by impaired endothelial cell function, astrocyte damage, and inflammatory cell activation can contribute to oligodendrocyte injury and demyelination. It has been proposed that extracellular vesicles (EVs) are possible triggers for this immune-mediated process. Various types of cells secrete individual EVs. Serum platelet EVs, fibrinogen EVs, glycolipid EVs, microglia/leukocyte EVs, and endothelial EVs, have been linked to MS pathology. Similarly, the high levels of microglia/leukocyte EVs and oligodendrocyte EVs in CSF have been related to MS. The elevation in CSF astrocyte EVs is associated with NMOSD. Alteration in both serum and CSF microRNAs is associated with demyelinating disorders. CD, cluster of differentiation; CLIC1, Chloride Intracellular Channel 1; CSF, cerebrospinal fluid; cxcl13, C-X-C Motif Chemokine Ligand 13; DSG2, Desmoglein 2; EV, extracellular vesicles; GFAP, Glial fibrillary acidic protein; GZMA, Granzyme A; ICAM1, Intercellular Adhesion Molecule 1; IgM, immunoglobulin M; miR, microribonucleic acid; MMP7, matrix metalloproteinase 7; MOG; myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; NMOSD, neuromyelitis optica spectrum disorder; PECAM, Platelet endothelial cell adhesion molecule; SERPINA3, Serpin Family A Member 3; TNFRSF25, TNF Receptor Superfamily Member 25.

and syncytiotrophoblasts. EVs generated by cytotrophoblasts and syncytiotrophoblasts enhance embryonic and placental development. Martire et al. demonstrated an association between placental EVs and MS patients by analyzing EVs secreted from placental tissue culture. Major EV groups, specifically embryonic, mesenchymal, and hematopoietic stem cells, showed high expressive capacity. EVs from these origins have been reported to inhibit the inflammatory cascade and enhance immune tolerance. Contrarily, the EVs derived from antigen-presenting cells (such as HLA-A/B/C molecules, CD86, and CD1c), T cells (CD2, CD3, CD25), activated immune cells with cytotoxic properties (CD56), and B cells (CD19, CD20) were significantly decreased in pregnant women [98]. Tumor necrosis factor (TNF)-related EVs, specifically Fas ligand (FasL) and TNF-related apoptosis-inducing ligand (TRAIL), suppressed inflammation by triggering apoptosis in peripheral mononuclear cells. Trophoblast-related EVs are also related to Treg proliferation and NF-κB activation inhibition [99]. Steroid hormone concentrations, which include estrogen and progesterone, surge in pregnant women, particularly in the third trimester. Progesterone at high concentrations causes lymphocytes to release progesterone-induced binding factor (PIBF). The findings demonstrated that PIBF continuously rises during pregnancy and declines dramatically after delivery. PIBF is associated with the secretion of anti-inflammatory cytokines from Th2 cells, including IL-4, IL-5, and IL-10 [98, 100]. In addition, EAE mice injected with placental EVs showed a decrease in T cell proliferation, increase

Table 5. Extracellular vesicles markers in central demyelinating diseases and therapeutic strategies: reports from *clinical studies*

Treatment	Design and population	Methods	Specimens	EV origins	Major findings in treated group	Interpretation	Ref
Fingolimod	Treated: 19 Control: Untreated MS 15, Healthy: 15	Scanning electron microscope, flow cytometry	Platelet-free plasma	Endothelium	↓CD105	Fingolimod reduced endothelial MP levels.	[93]
Fingolimod	Treated: 11 Untreated: 5 Healthy: 8	Nanoparticle tracking system, cryo-electron microscopy, flow cytometry	plasma	Platelet Leukocyte Monocyte	↑EV concentration at 5 hours ↓Lymphocyte activation at 5 hours	Fingolimod inhibited EV immediately after initiation and activated compensatory mechanisms, resulting in increased EV at 5 hours.	[92]
IFN-β	Treated: 7 Untreated: 4	qPCR	Serum	NA	↑miR-22-3p ↑miR-660-5p ↓miR-486-5p ↓miR-451a ↓let-7b-5p ↓miR-320b ↓miR-122-5p ↓miR-215-5p ↓miR-320d ↓miR-19-3p ↓miR-26a-5p ↓miR-142-3p ↓miR-146a-5p ↓miR-15b-3p ↓miR-23a-3p ↓miR-223-3p	Many inflammatory related exosomes altered after IFN-β treatment.	[91]
IFN-β	Treated: 20 Untreated: 24	Flow cytometry	blood	Platelet Leukocyte	↑CD61 (PMP) ↑CD45 (LMP) ↑CD14 (LMP)	IFN-β increased PMP and LMP.	[42]
IFN-β1a	Treated: 30 Untreated: 79	Flow cytometry	Platelet-poor plasma	Endothelium	↓CD31+ (EMP)	IFN-β1a decreased EMP.	[89]
IFN-β1a	Treated: 16	Flow cytometry	plasma	Endothelium	↓CD31+ at 3, 6, 12 months ↓CD146+ at 3 months ↓CD54+ at 3, 6, 12 months ↓T2-weighted lesion volume	CD31+ and CD54+ could be used as surrogate markers for IFN-β1a treatment.	[39]
IFN-β1b	Comparing before and after treatment: 11 relapsing MS, 9 remitting MS Healthy: 10	Cultured BMVEC with EMP and detection by flow cytometry from	plasma	Endothelium	↓CD31 ↓CD54 ↓CD62E ↓Transmigration of monocyte ↓Transmigration of monocyte:EMP	IFN-β1b inhibited the adhesion of lymphocytes and endothelium and prevented T cells entering BBB.	[90]
Natalizumab	Treated: 20 Untreated: 24	Flow cytometry	blood	Platelet Leukocyte	↑CD61 (PMP) ↑CD45 (LMP) ↑CD14 (LMP)	Natalizumab increased PMP and LMP.	[42]

ASMase, acid sphingomyelinase; BBB, blood brain barrier; BMVEC; brain microvascular endothelial cells; EV, extracellular vesicles; HC, healthy control; IFN-β, interferon-beta; EMP, endothelial microparticle; LMP, leukocyte microparticle; miR, microribonucleic acid; MP, microparticle; MS, multiple sclerosis; NA, not available; PBMC, peripheral blood mononuclear cells; PMP, platelet microparticle; qPCR, quantitative polymerase chain reactio.

Treg cell, and a subsequent decrease in inflammation adding weight to these findings [101, 102]. The same effects were observed in EVs produced from bone marrow, periodontal ligament cells, adipose tissue, and umbilical cord mesenchyme, which raised the levels of protective cytokines, such as IL10, transforming growth factor-beta (TGF-β), and stromal cell-derived factor 1 alpha (SDF-1α), but reduced pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α) and NALP-3 [94, 96, 103-109].

The effectiveness of mesenchymal EVs has not only been observed in human-derived mesenchymal EVs, but also in EAE mice treated with Rhesus monkey mesenchymal stromal cell EVs. This treatment showed evidence of stimulation of oligodendrocytes and prevention of the polarization of M2 to M1 microglia, resulting in remyelination [95]. EVs from doxycycline-inducible let-7g transgenic (Let-7Tg) mice also resulted in measurable benefits with regard to inflammatory suppression. Let-7 inhibits CD4+ T cell proliferation, which may contribute to the lower number of CNS-infiltrated Let-7Tg CD4+ T cells. Th17 differentiation and IL1 receptor 1 (IL1R1) activity were suppressed by the Let-7g transgene, resulting in a pro-regenerative environment [110].

Since the goal of improving MS demyelination is to promote remyelination, many EVs were examined to investigate their efficacy with regard to possible effects [111-113]. Intranasally administered miR-219a-5p extracts boosted OPC proliferation in EAE mice resulting in remyelination [114]. IFN-stimulated dendritic cell EV aided remyelination, as indicated by an increase in MBP. One study found that the exposure of aging animals to EV-containing serum from youth animals also increases oligodendrocyte proliferation and promotes remyelination [115]. The experimental model discovered that environmentally enriched EVs primarily containing miR-219 can promote remyelination, but miR-219 deficient EVs cause remyelination failure and inhibit oligodendrocyte progenitor cell proliferation [116, 117]. An *in vitro* study showed that IL4 promotes remyelination by causing microglia to transform into the pro-regenerative form [53]. All of these findings suggest that personalized medicine is possible through the development of EVs as carrier for numerous therapeutic compounds for MS. All data is summarized in Table 6. Potential interventions are also illustrated in Fig. 2.

CONCLUSION

EVs have been shown to be linked to the pathogenesis of immune-mediated central demyelinating disorders, however the

https://doi.org/10.5607/en24002

Table 6. Extracellular vesicles markers in central demyelinating diseases and therapeutic strategy: reports from *in vivo* and *in vitro* studies

Treatment	Design and population	EV origins	Major finding in treated group	Interpretation	Ref
In vivo studies					
Placental exosomes	EAE mice with placental EV injection	Human placenta	↓ T cell proliferation	Placental EV suppressed T cell proliferation and inflammation.	[101]
	EAE mice injected with mice placental EV	Mice placenta	↑ Treg	Placental EV promote Treg population resulting in anti-inflammatory state	[102]
Human MSC	EAE mice injected with supernatant of cultured BMSC	Bone marrow	↑ IL10 ↑ TGFβ ↓ TNFα	BMSC inhibited inflammatory process	[94]
	EAE mice injected with BMSC containing miR-367-3p	Bone marrow	↑ SLC7A11 ↑ GPX4 ↓ ferroptosis	BMSC-derived EVs suppress ferroptosis	[104]
	EAE mice injected with BMSC containing miR-23b-3p	Bone marrow	↓ IL17 ↓ Microglia inflammation	BMSC inhibited inflammatory process	[105]
	EAE mice injected with BMSC	Bone marrow	↑ Anti-inflammation: miR-193, miR-146a ↓ Proinflammation: miR-155, miR-21, and miR-326	BMSC inhibited inflammatory process	[108]
	EAE mice injected with Chimiric TAxI-peptide-chimeric Umbilical cord MSC-exos	Chimeric Umbilical cord MSC	↑ Anti-inflammation: IL-4, IL-10, TGF-β, IDO-1 ↓ proinflammation: IL-2, IL-6, IL-17A, IFN-γ, and TNF-α	Chimeric umbilical cord MSCs can suppress inflammatory process of MS	[106]
	EAE mice injected with Umblical cord MSC containing miR-23a-3p	Umbilical cord MSC	↑ OPC	Umbilical cord MSC can promote remyelination in MS model	[107]
Periodontal ligament stem cell exosomes	EAE mice injected with hPDLSC	Periodontal ligaments	↑ IL10 ↑ TGFβ ↑ SDF-1α ↓ NALP3	hPDLSC inhibited inflammatory process.	[103]
Rhesus monkey MSC	EAE mice injected with MSC	Mesenchymal cells	↑ MBP ↓ Amyloid-β precursor protein ↑ M2 microglia ↓ M1 microglia	MSC promoted remyelination by promoted oligodendrocyte and inhibited inflammatory microglia.	[95]
Mouse MSC	EAE mice injected with mouse MSC loaded with FOX3P gene	Bone marrow	↑ Treg ↑ IL10	BSC EV promote Treg population and function resulting in anti-inflammatory.	[111]
M2 macrophage derived exosomes	EAE mice injected with si-lncRNA PVT1	M2 macrophages	↑ Treg activity ↓ Th17	lncRNA PVT1 could suppress inflammation of RRMS.	[112]
	EAE mice injected with miR-21-5p	M2 macrophages	↓ Th17	miR-21-5p caused Th17 differentiation silencing.	
miR-219a-5p exosomes	EAE mice intranasally injected with miR-219a-5p	miR-219a-5p	↑ OPC	miR-219a-5p promoted remyelination.	[114]
Let-7	EAE mice with Let-7g transgene	Let-7	↓ Th17 differentiation ↓ IL1R1 ↓ IL23R ↓ CCR2 ↓ CCR5	Let-7 exosomes reduced Th17 differentiation by blocking IL1R1 and IL23R and inhibition of CCR3 and CCR5.	[110]
IFNγ-stimulated dendritic cells	Intranasal administration of IFNγ-stimulated DC exosome with lysolecithin injection	Dendritic cells	↑ MBP	IFNγ-stimulated dendritic cell exosomes associated with remyelination.	[116]
Environmental enrichment exosomes	Serum from enriched rat cultured with rat hippocampus with lysolecithin injection	Blood exosome	↑ MBP	Serum environmental enriched exosomes associated with remyelination.	[117]
In vitro studies					
IL4	In vitro study: Microglia cultured with OPC IL4	IL4	↑ Argenase 1 ↑ MBP ↑ OPC accumulation	Pro-regenerative exposed microglia induced remyelination	[53]
MSC	Umbilical cord MSC and their exosome co-culture with PBMC from MS patients and HC. EV detected by flow cytometry	Mesenchymal stem cells	↓ PBMC proliferation	MSCs inhibited mononuclear cell proliferation.	[96]
	Umbilical cord MSC and their exosome co-cultured with T cells	Umbilical cord mesenchymal stem cells	↑ IL10	MSCs induced anti-inflammatory IL-10 secreting T cell.	[113]
	Adipose tissue MSC containing miR-29b co-cultured with T cells	Adipose tissue	↓ IL-4 ↓ IL-17 ↓ Tbx21 ↓ RORγt	Adipose tissue MSC associated with T cell differentiation into anti-inflammatory T cells.	[109]
IFNγ-stimulated DC	IFNγ-stimulated DC exosome cultured with mice hippocampus	Dendritic cells	↑ miR-219 ↑ MBP ↓ Reduced glutathione ↓ PDGFRα ↓ ELOVL7	IFNγ-stimulated DC exosomes mainly contained miR-219 and were associated with remyelination.	[116]
Environmental enrichment exosomes	Environmental enrichment exosomes cultured with rat hippocampus	Serum with Environmental enrichment	↑ MBP ↑ miR-219	Environmental enriched exosomes mainly contained miR-219 and associated with remyelination.	[117]

BMSC, bone marrow stem cells; CCR2, C-C Motif Chemokine Receptor 2; CCR5, C-C Motif Chemokine Receptor 5; DC, dendritic cells; EAE, experimental autoimmune encephalitis; ELOVL7, fatty acid elongase 7; EV, extracellular vesicles; hPDLSC, human periodontal ligament stem cells exosome; let-7, lethal-7; IFNγ, interferon gamma; IL, interleukin; IL1R1, Interleukin 1 receptor Type 1;IL23R, Interleukin 23 receptor; lncRNA, long non-coding ribonucleic acid; iNOS, inducible nitric oxide synthase; MBP, myelin basic protein; MHC II, major histocompatibility complex class 2; miR, microribonucleic acid; MSC, mesenchymal cells; NALP3, NLR family pyrin domain containing 3; OPC, oligodendrocyte precursor cell; PDGFRα, platelet derived growth factor receptor alpha; PLP, proteolipid protein; PVT1, Plasmacytoma Variant Translocation 1; RRMS, relapsing remitting multiple sclerosis; SDF-1α, stromal cell-derived factor-1 alpha; si-lncRNA, silencing long non-coding ribonucleic acid; TGFβ, transforming growth factor beta; Th17, T helper 17 cells; TNFα, tumor necrosis factor alpha; Treg, regulatory T cells.

actions and presence of individual EVs have been found to vary between different diseases. Platelet and endothelial EVs, as well as activated microglia-derived exosomes, were found to be associated with synaptic and BBB dysfunctions in MS. *In vivo* data provided evidence of an association between fibrinogen and astrocyte-derived exosomes with neuroinflammation and demyelination. Although robust evidence from serum and CSF of MS individuals highlighted the role of various EVs, including those from leukocytes, microglia, and microRNAs, concerning central inflammatory pathways, there was still no definitive evidence regarding the implementation of EVs as a surrogate biomarker in diagnosis, prognosis management or therapeutic approaches. NMOSDs are

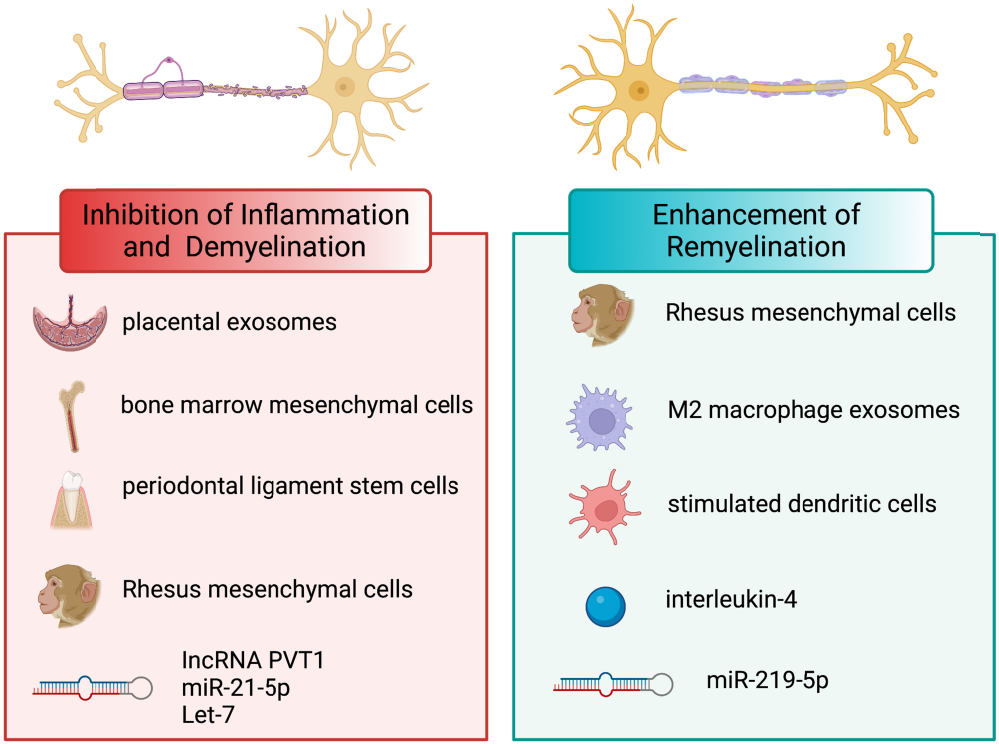

Fig. 2. Potential therapeutic interventions in immune-mediated central demyelinating diseases. The therapeutic goals in demyelinating diseases consist of two components: demyelination inhibition and remyelination enhancement. Mesenchymal stem cells from the placenta, bone marrow, and periodontal ligaments have been shown to reduce inflammation and demyelination. However, EVs from macrophages, dendritic cells and synthetic interleukin-4 can stimulate the remyelinating process. Interestingly, Rhesus monkey mesenchymal cells show both demyelinating inhibition and remyelinating promotion. let-7, lethal-7; lncRNA, long non-coding ribonucleic acid; miR, microribonucleic acid.

a new spectrum of demyelinating diseases that to date lack data concerning the role of EVs. There is limited clinical evidence to indicate the difference in the action of exosomes between NMOSD and MS, however some miRNA dysregulation has been shown to be related to clinical relapses of those diseases. Even though MS and NMOSD are considered CDD and share overlapping clinical features the data pertinent to EVs and CDD mostly focus on MS, whereas the data on NMOSD remain limited. Increased research and data into the clinical use of EVs as a potential therapeutic strategy is still required. However, as they have been considered carriers for diverse biological molecules, there is a very real possibility that EVs could be exploited as a next-generation targeted therapy as a precision medicine approach for MS and NMOSD.

FUNDING

This work was supported by the Distinguished Research Professor grant from the National Research Council of Thailand (N42A660301 to SCC); Research Chair Grant from the National Research Council of Thailand (NC) and a Chiang Mai University Center of Excellence Award (NC).

CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

REFERENCES

1. Filippi M, Bar-Or A, Piehl F, Preziosa P, Solari A, Vukusic S, Rocca MA (2018) Multiple sclerosis. Nat Rev Dis Primers 4:43.
2. Ghasemi N, Razavi S, Nikzad E (2017) Multiple sclerosis: pathogenesis, symptoms, diagnoses and cell-based therapy. Cell J 19:1-10.
3. Loma I, Heyman R (2011) Multiple sclerosis: pathogenesis and treatment. Curr Neuropharmacol 9:409-416.
4. Wosik K, Antel J, Kuhlmann T, Brück W, Massie B, Nalbantoglu J (2003) Oligodendrocyte injury in multiple sclerosis: a role for p53. J Neurochem 85:635-644.
5. Steinman L (2014) Immunology of relapse and remission in multiple sclerosis. Annu Rev Immunol 32:257-281.
6. Dendrou CA, Fugger L, Friese MA (2015) Immunopathology

of multiple sclerosis. Nat Rev Immunol 15:545-558.
7. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, Correale J, Fazekas F, Filippi M, Freedman MS, Fujihara K, Galetta SL, Hartung HP, Kappos L, Lublin FD, Marrie RA, Miller AE, Miller DH, Montalban X, Mowry EM, Sorensen PS, Tintoré M, Traboulsee AL, Trojano M, Uitdehaag BMJ, Vukusic S, Waubant E, Weinshenker BG, Reingold SC, Cohen JA (2018) Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 17:162-173.
8. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR (2005) IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. J Exp Med 202:473-477.
9. Jarius S, Paul F, Weinshenker BG, Levy M, Kim HJ, Wildemann B (2020) Neuromyelitis optica. Nat Rev Dis Primers 6:85.
10. Weinshenker BG, Wingerchuk DM (2017) Neuromyelitis spectrum disorders. Mayo Clin Proc 92:663-679.
11. Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, de Seze J, Fujihara K, Greenberg B, Jacob A, Jarius S, Lana-Peixoto M, Levy M, Simon JH, Tenembaum S, Traboulsee AL, Waters P, Wellik KE, Weinshenker BG; International Panel for NMO Diagnosis (2015) International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology 85:177-189.
12. Bennett JL, Aktas O, Rees WA, Smith MA, Gunsior M, Yan L, She D, Cimbora D, Pittock SJ, Weinshenker BG, Paul F, Marignier R, Wingerchuk D, Cutter G, Green A, Hartung HP, Kim HJ, Fujihara K, Levy M, Katz E, Cree BAC; N-MOmentum study investigators (2022) Association between B-cell depletion and attack risk in neuromyelitis optica spectrum disorder: an exploratory analysis from N-MOmentum, a double-blind, randomised, placebo-controlled, multicentre phase 2/3 trial. EBioMedicine 86:104321.
13. Fenoglio C, Ridolfi E, Galimberti D, Scarpini E (2012) MicroRNAs as active players in the pathogenesis of multiple sclerosis. Int J Mol Sci 13:13227-13239.
14. Urabe F, Kosaka N, Ito K, Kimura T, Egawa S, Ochiya T (2020) Extracellular vesicles as biomarkers and therapeutic targets for cancer. Am J Physiol Cell Physiol 318:C29-C39.
15. Lee EC, Ha TW, Lee DH, Hong DY, Park SW, Lee JY, Lee MR, Oh JS (2022) Utility of exosomes in ischemic and hemorrhagic stroke diagnosis and treatment. Int J Mol Sci 23:8367.
16. DeLeo AM, Ikezu T (2018) Extracellular vesicle biology in Alzheimer's disease and related tauopathy. J Neuroimmune Pharmacol 13:292-308.
17. Hill AF (2019) Extracellular vesicles and neurodegenerative diseases. J Neurosci 39:9269-9273.
18. Zhou B, Xu K, Zheng X, Chen T, Wang J, Song Y, Shao Y, Zheng S (2020) Application of exosomes as liquid biopsy in clinical diagnosis. Signal Transduct Target Ther 5:144.
19. Lapitz A, Azkargorta M, Milkiewicz P, Olaizola P, Zhuravleva E, Grimsrud MM, Schramm C, Arbelaiz A, O'Rourke CJ, La Casta A, Milkiewicz M, Pastor T, Vesterhus M, Jimenez-Agüero R, Dill MT, Lamarca A, Valle JW, Macias RIR, Izquierdo-Sanchez L, Pérez Castaño Y, Caballero-Camino FJ, Riaño I, Krawczyk M, Ibarra C, Bustamante J, Nova-Camacho LM, Falcon-Perez JM, Elortza F, Perugorria MJ, Andersen JB, Bujanda L, Karlsen TH, Folseraas T, Rodrigues PM, Banales JM (2023) Liquid biopsy-based protein biomarkers for risk prediction, early diagnosis, and prognostication of cholangiocarcinoma. J Hepatol 79:93-108.
20. Cheng L, Vella LJ, Barnham KJ, McLean C, Masters CL, Hill AF (2020) Small RNA fingerprinting of Alzheimer's disease frontal cortex extracellular vesicles and their comparison with peripheral extracellular vesicles. J Extracell Vesicles 9:1766822.
21. Kanchanapally R, Deshmukh SK, Chavva SR, Tyagi N, Srivastava SK, Patel GK, Singh AP, Singh S (2019) Drug-loaded exosomal preparations from different cell types exhibit distinctive loading capability, yield, and antitumor efficacies: a comparative analysis. Int J Nanomedicine 14:531-541.
22. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK, Ayre DC, Bach JM, Bachurski D, Baharvand H, Balaj L, Baldacchino S, Bauer NN, Baxter AA, Bebawy M, Beckham C, Bedina Zavec A, Benmoussa A, Berardi AC, Bergese P, Bielska E, Blenkiron C, Bobis-Wozowicz S, Boilard E, Boireau W, Bongiovanni A, Borràs FE, Bosch S, Boulanger CM, Breakefield X, Breglio AM, Brennan MÁ, Brigstock DR, Brisson A, Broekman ML, Bromberg JF, Bryl-Górecka P, Buch S, Buck AH, Burger D, Busatto S, Buschmann D, Bussolati B, Buzás EI, Byrd JB, Camussi G, Carter DR, Caruso S, Chamley LW, Chang YT, Chen C, Chen S, Cheng L, Chin AR, Clayton A, Clerici SP, Cocks A, Cocucci E, Coffey RJ, Cordeiro-da-Silva A, Couch Y, Coumans FA, Coyle B, Crescitelli R, Criado MF, D'Souza-Schorey C, Das S, Datta Chaudhuri A, de Candia P, De Santana EF, De Wever O, Del Portillo HA, Demaret T, Deville S, Devitt A, Dhondt B, Di Vizio D, Dieterich LC, Dolo V, Dominguez Rubio AP, Dominici M, Dourado MR, Driedonks TA, Duarte FV, Duncan HM, Eichenberger RM, Ekström K, El Andaloussi S, Elie-Caille C, Erdbrügger U, Falcón-Pérez JM, Fatima F, Fish JE, Flores-Bellver M, Försönits A, Frelet-Barrand A, Fricke F, Fuhrmann G, Gabrielsson S, Gámez-Valero A, Gardiner C, Gärtner K, Gaudin R, Gho YS, Giebel B, Gilbert C, Gimona M, Giusti I, Goberdhan DC,

Görgens A, Gorski SM, Greening DW, Gross JC, Gualerzi A, Gupta GN, Gustafson D, Handberg A, Haraszti RA, Harrison P, Hegyesi H, Hendrix A, Hill AF, Hochberg FH, Hoffmann KF, Holder B, Holthofer H, Hosseinkhani B, Hu G, Huang Y, Huber V, Hunt S, Ibrahim AG, Ikezu T, Inal JM, Isin M, Ivanova A, Jackson HK, Jacobsen S, Jay SM, Jayachandran M, Jenster G, Jiang L, Johnson SM, Jones JC, Jong A, Jovanovic-Talisman T, Jung S, Kalluri R, Kano SI, Kaur S, Kawamura Y, Keller ET, Khamari D, Khomyakova E, Khvorova A, Kierulf P, Kim KP, Kislinger T, Klingeborn M, Klinke DJ 2nd, Kornek M, Kosanović MM, Kovács ÁF, Krämer-Albers EM, Krasemann S, Krause M, Kurochkin IV, Kusuma GD, Kuypers S, Laitinen S, Langevin SM, Languino LR, Lannigan J, Lässer C, Laurent LC, Lavieu G, Lázaro-Ibáñez E, Le Lay S, Lee MS, Lee YXF, Lemos DS, Lenassi M, Leszczynska A, Li IT, Liao K, Libregts SF, Ligeti E, Lim R, Lim SK, Linē A, Linnemannstöns K, Llorente A, Lombard CA, Lorenowicz MJ, Lörincz ÁM, Lötvall J, Lovett J, Lowry MC, Loyer X, Lu Q, Lukomska B, Lunavat TR, Maas SL, Malhi H, Marcilla A, Mariani J, Mariscal J, Martens-Uzunova ES, Martin-Jaular L, Martinez MC, Martins VR, Mathieu M, Mathivanan S, Maugeri M, McGinnis LK, McVey MJ, Meckes DG Jr, Meehan KL, Mertens I, Minciacchi VR, Möller A, Møller Jørgensen M, Morales-Kastresana A, Morhayim J, Mullier F, Muraca M, Musante L, Mussack V, Muth DC, Myburgh KH, Najrana T, Nawaz M, Nazarenko I, Nejsum P, Neri C, Neri T, Nieuwland R, Nimrichter L, Nolan JP, Nolte-'t Hoen EN, Noren Hooten N, O'Driscoll L, O'Grady T, O'Loghlen A, Ochiya T, Olivier M, Ortiz A, Ortiz LA, Osteikoetxea X, Østergaard O, Ostrowski M, Park J, Pegtel DM, Peinado H, Perut F, Pfaffl MW, Phinney DG, Pieters BC, Pink RC, Pisetsky DS, Pogge von Strandmann E, Polakovicova I, Poon IK, Powell BH, Prada I, Pulliam L, Quesenberry P, Radeghieri A, Raffai RL, Raimondo S, Rak J, Ramirez MI, Raposo G, Rayyan MS, Regev-Rudzki N, Ricklefs FL, Robbins PD, Roberts DD, Rodrigues SC, Rohde E, Rome S, Rouschop KM, Rughetti A, Russell AE, Saá P, Sahoo S, Salas-Huenuleo E, Sánchez C, Saugstad JA, Saul MJ, Schiffelers RM, Schneider R, Schøyen TH, Scott A, Shahaj E, Sharma S, Shatnyeva O, Shekari F, Shelke GV, Shetty AK, Shiba K, Siljander PR, Silva AM, Skowronek A, Snyder OL 2nd, Soares RP, Sódar BW, Soekmadji C, Sotillo J, Stahl PD, Stoorvogel W, Stott SL, Strasser EF, Swift S, Tahara H, Tewari M, Timms K, Tiwari S, Tixeira R, Tkach M, Toh WS, Tomasini R, Torrecilhas AC, Tosar JP, Toxavidis V, Urbanelli L, Vader P, van Balkom BW, van der Grein SG, Van Deun J, van Herwijnen MJ, Van Keuren-Jensen K, van Niel G, van Royen ME, van Wijnen AJ, Vasconcelos MH, Vechetti IJ Jr, Veit TD, Vella LJ, Velot É, Verweij FJ, Vestad B, Viñas JL, Visnovitz T, Vukman KV, Wahlgren J, Watson DC, Wauben MH, Weaver A, Webber JP, Weber V, Wehman AM, Weiss DJ, Welsh JA, Wendt S, Wheelock AM, Wiener Z, Witte L, Wolfram J, Xagorari A, Xander P, Xu J, Yan X, Yáñez-Mó M, Yin H, Yuana Y, Zappulli V, Zarubova J, Žėkas V, Zhang JY, Zhao Z, Zheng L, Zheutlin AR, Zickler AM, Zimmermann P, Zivkovic AM, Zocco D, Zuba-Surma EK (2018) Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 7:1535750.

23. Doyle LM, Wang MZ (2019) Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. Cells 8:727.
24. Cocucci E, Meldolesi J (2015) Ectosomes and exosomes: shedding the confusion between extracellular vesicles. Trends Cell Biol 25:364-372.
25. Cocucci E, Racchetti G, Meldolesi J (2009) Shedding microvesicles: artefacts no more. Trends Cell Biol 19:43-51.
26. Bergsmedh A, Szeles A, Henriksson M, Bratt A, Folkman MJ, Spetz AL, Holmgren L (2001) Horizontal transfer of oncogenes by uptake of apoptotic bodies. Proc Natl Acad Sci U S A 98:6407-6411.
27. Wu X, Liu Y, Wei W, Liu ML (2019) Extracellular vesicles in autoimmune vasculitis - little dirts light the fire in blood vessels. Autoimmun Rev 18:593-606.
28. Gardiner C, Di Vizio D, Sahoo S, Théry C, Witwer KW, Wauben M, Hill AF (2016) Techniques used for the isolation and characterization of extracellular vesicles: results of a worldwide survey. J Extracell Vesicles 5:32945.
29. Arraud N, Linares R, Tan S, Gounou C, Pasquet JM, Mornet S, Brisson AR (2014) Extracellular vesicles from blood plasma: determination of their morphology, size, phenotype and concentration. J Thromb Haemost 12:614-627.
30. Saugstad JA, Lusardi TA, Van Keuren-Jensen KR, Phillips JI, Lind B, Harrington CA, McFarland TJ, Courtright AL, Reiman RA, Yeri AS, Kalani MYS, Adelson PD, Arango J, Nolan JP, Duggan E, Messer K, Akers JC, Galasko DR, Quinn JF, Carter BS, Hochberg FH (2017) Analysis of extracellular RNA in cerebrospinal fluid. J Extracell Vesicles 6:1317577.
31. Skalnikova HK, Bohuslavova B, Turnovcova K, Juhasova J, Juhas S, Rodinova M, Vodicka P (2019) Isolation and characterization of small extracellular vesicles from porcine blood plasma, cerebrospinal fluid, and seminal plasma. Proteomes 7:17.
32. Veerman RE, Teeuwen L, Czarnewski P, Güclüler Akpinar G, Sandberg A, Cao X, Pernemalm M, Orre LM, Gabrielsson S, Eldh M (2021) Molecular evaluation of five different isola-

tion methods for extracellular vesicles reveals different clinical applicability and subcellular origin. J Extracell Vesicles 10:e12128.
33. Marcos-Ramiro B, Oliva Nacarino P, Serrano-Pertierra E, Blanco-Gelaz MA, Weksler BB, Romero IA, Couraud PO, Tuñón A, López-Larrea C, Millán J, Cernuda-Morollón E (2014) Microparticles in multiple sclerosis and clinically isolated syndrome: effect on endothelial barrier function. BMC Neurosci 15:110.
34. Minagar A, Jy W, Jimenez JJ, Sheremata WA, Mauro LM, Mao WW, Horstman LL, Ahn YS (2001) Elevated plasma endothelial microparticles in multiple sclerosis. Neurology 56:1319-1324.
35. Alexander JS, Chervenak R, Weinstock-Guttman B, Tsunoda I, Ramanathan M, Martinez N, Omura S, Sato F, Chaitanya GV, Minagar A, McGee J, Jennings MH, Monceaux C, Becker F, Cvek U, Trutschl M, Zivadinov R (2015) Blood circulating microparticle species in relapsing-remitting and secondary progressive multiple sclerosis. A case-control, cross sectional study with conventional MRI and advanced iron content imaging outcomes. J Neurol Sci 355:84-89.
36. Jy W, Minagar A, Jimenez JJ, Sheremata WA, Mauro LM, Horstman LL, Bidot C, Ahn YS (2004) Endothelial microparticles (EMP) bind and activate monocytes: elevated EMP-monocyte conjugates in multiple sclerosis. Front Biosci 9:3137-3144.
37. Wheway J, Latham SL, Combes V, Grau GE (2014) Endothelial microparticles interact with and support the proliferation of T cells. J Immunol 193:3378-3387.
38. Achen MG, Clauss M, Schnürch H, Risau W (1995) The non-receptor tyrosine kinase Lyn is localised in the developing murine blood-brain barrier. Differentiation 59:15-24.
39. Lowery-Nordberg M, Eaton E, Gonzalez-Toledo E, Harris MK, Chalamidas K, McGee-Brown J, Ganta CV, Minagar A, Cousineau D, Alexander JS (2011) The effects of high dose interferon-β1a on plasma microparticles: correlation with MRI parameters. J Neuroinflammation 8:43.
40. Smyth SS, McEver RP, Weyrich AS, Morrell CN, Hoffman MR, Arepally GM, French PA, Dauerman HL, Becker RC; 2009 Platelet Colloquium Participants (2009) Platelet functions beyond hemostasis. J Thromb Haemost 7:1759-1766.
41. Kawachi I, Lassmann H (2017) Neurodegeneration in multiple sclerosis and neuromyelitis optica. J Neurol Neurosurg Psychiatry 88:137-145.
42. Sáenz-Cuesta M, Irizar H, Castillo-Triviño T, Muñoz-Culla M, Osorio-Querejeta I, Prada A, Sepúlveda L, López-Mato MP, López de Munain A, Comabella M, Villar LM, Olascoaga J, Otaegui D (2014) Circulating microparticles reflect treatment effects and clinical status in multiple sclerosis. Biomark Med 8:653-661.
43. Sheremata WA, Jy W, Horstman LL, Ahn YS, Alexander JS, Minagar A (2008) Evidence of platelet activation in multiple sclerosis. J Neuroinflammation 5:27.
44. Geraci F, Ragonese P, Barreca MM, Aliotta E, Mazzola MA, Realmuto S, Vazzoler G, Savettieri G, Sconzo G, Salemi G (2018) Differences in intercellular communication during clinical relapse and gadolinium-enhanced MRI in patients with relapsing remitting multiple sclerosis: a study of the composition of extracellular vesicles in cerebrospinal fluid. Front Cell Neurosci 12:418.
45. Quandt J, Dorovini-Zis K (2004) The beta chemokines CCL4 and CCL5 enhance adhesion of specific CD4+ T cell subsets to human brain endothelial cells. J Neuropathol Exp Neurol 63:350-362.
46. Szpakowski P, Ksiazek-Winiarek D, Czpakowska J, Kaluza M, Milewska-Jedrzejczak M, Glabinski A (2023) Astrocyte-derived exosomes differentially shape T cells' immune response in MS patients. Int J Mol Sci 24:7470.
47. Benjamins JA, Nedelkoska L, Touil H, Stemmer PM, Carruthers NJ, Jena BP, Naik AR, Bar-Or A, Lisak RP (2019) Exosome-enriched fractions from MS B cells induce oligodendrocyte death. Neurol Neuroimmunol Neuroinflamm 6:e550.
48. Torres Iglesias G, Fernández-Fournier M, Botella L, Piniella D, Laso-García F, Carmen Gómez-de Frutos M, Chamorro B, Puertas I, Tallón Barranco A, Fuentes B, Alonso de Leciñana M, Alonso-López E, Bravo SB, Eugenia Miranda-Carús M, Montero-Calle A, Barderas R, Díez-Tejedor E, Gutiérrez-Fernández M, Otero-Ortega L (2023) Brain and immune system-derived extracellular vesicles mediate regulation of complement system, extracellular matrix remodeling, brain repair and antigen tolerance in Multiple sclerosis. Brain Behav Immun 113:44-55.
49. Azimi M, Ghabaee M, Moghadasi AN, Noorbakhsh F, Izad M (2018) Immunomodulatory function of Treg-derived exosomes is impaired in patients with relapsing-remitting multiple sclerosis. Immunol Res 66:513-520.
50. Masvekar R, Wu T, Kosa P, Barbour C, Fossati V, Bielekova B (2019) Cerebrospinal fluid biomarkers link toxic astrogliosis and microglial activation to multiple sclerosis severity. Mult Scler Relat Disord 28:34-43.
51. Verderio C, Muzio L, Turola E, Bergami A, Novellino L, Ruffini F, Riganti L, Corradini I, Francolini M, Garzetti L, Maiorino C, Servida F, Vercelli A, Rocca M, Dalla Libera D,

Martinelli V, Comi G, Martino G, Matteoli M, Furlan R (2012) Myeloid microvesicles are a marker and therapeutic target for neuroinflammation. Ann Neurol 72:610-624.

52. Prada I, Gabrielli M, Turola E, Iorio A, D'Arrigo G, Parolisi R, De Luca M, Pacifici M, Bastoni M, Lombardi M, Legname G, Cojoc D, Buffo A, Furlan R, Peruzzi F, Verderio C (2018) Glia-to-neuron transfer of miRNAs via extracellular vesicles: a new mechanism underlying inflammation-induced synaptic alterations. Acta Neuropathol 135:529-550.
53. Lombardi M, Parolisi R, Scaroni F, Bonfanti E, Gualerzi A, Gabrielli M, Kerlero de Rosbo N, Uccelli A, Giussani P, Viani P, Garlanda C, Abbracchio MP, Chaabane L, Buffo A, Fumagalli M, Verderio C (2019) Detrimental and protective action of microglial extracellular vesicles on myelin lesions: astrocyte involvement in remyelination failure. Acta Neuropathol 138:987-1012.
54. Galazka G, Mycko MP, Selmaj I, Raine CS, Selmaj KW (2018) Multiple sclerosis: serum-derived exosomes express myelin proteins. Mult Scler 24:449-458.
55. Agliardi C, Guerini FR, Zanzottera M, Bolognesi E, Picciolini S, Caputo D, Rovaris M, Pasanisi MB, Clerici M (2023) Myelin basic protein in oligodendrocyte-derived extracellular vesicles as a diagnostic and prognostic biomarker in multiple sclerosis: a pilot study. Int J Mol Sci 24:894.
56. Willis CM, Ménoret A, Jellison ER, Nicaise AM, Vella AT, Crocker SJ (2017) A refined bead-free method to identify astrocytic exosomes in primary glial cultures and blood plasma. Front Neurosci 11:335.
57. Petersen MA, Ryu JK, Akassoglou K (2018) Fibrinogen in neurological diseases: mechanisms, imaging and therapeutics. Nat Rev Neurosci 19:283-301.
58. Willis CM, Nicaise AM, Menoret A, Ryu JK, Mendiola AS, Jellison ER, Givogri MI, Han DK, Bongarzone ER, Akassoglou K, Vella AT, Crocker SJ (2019) Extracellular vesicle fibrinogen induces encephalitogenic CD8+ T cells in a mouse model of multiple sclerosis. Proc Natl Acad Sci U S A 116:10488-10493.
59. Admyre C, Johansson SM, Paulie S, Gabrielsson S (2006) Direct exosome stimulation of peripheral human T cells detected by ELISPOT. Eur J Immunol 36:1772-1781.
60. Mycko MP, Sliwinska B, Cichalewska M, Cwiklinska H, Raine CS, Selmaj KW (2014) Brain glycolipids suppress T helper cells and inhibit autoimmune demyelination. J Neurosci 34:8646-8658.
61. Maricic I, Sheng H, Marrero I, Seki E, Kisseleva T, Chaturvedi S, Molle N, Mathews SA, Gao B, Kumar V (2015) Inhibition of type I natural killer T cells by retinoids or following sulfatide-mediated activation of type II natural killer T cells attenuates alcoholic liver disease in mice. Hepatology 61:1357-1369.
62. Moyano AL, Li G, Boullerne AI, Feinstein DL, Hartman E, Skias D, Balavanov R, van Breemen RB, Bongarzone ER, Månsson JE, Givogri MI (2016) Sulfatides in extracellular vesicles isolated from plasma of multiple sclerosis patients. J Neurosci Res 94:1579-1587.
63. Muindi K, Cernadas M, Watts GF, Royle L, Neville DC, Dwek RA, Besra GS, Rudd PM, Butters TD, Brenner MB (2010) Activation state and intracellular trafficking contribute to the repertoire of endogenous glycosphingolipids presented by CD1d [corrected]. Proc Natl Acad Sci U S A 107:3052-3057.
64. Pieragostino D, Cicalini I, Lanuti P, Ercolino E, di Ioia M, Zucchelli M, Zappacosta R, Miscia S, Marchisio M, Sacchetta P, Onofrj M, Del Boccio P (2018) Enhanced release of acid sphingomyelinase-enriched exosomes generates a lipidomics signature in CSF of Multiple Sclerosis patients. Sci Rep 8:3071.
65. Welton JL, Loveless S, Stone T, von Ruhland C, Robertson NP, Clayton A (2017) Cerebrospinal fluid extracellular vesicle enrichment for protein biomarker discovery in neurological disease; multiple sclerosis. J Extracell Vesicles 6:1369805.
66. van Niel G, Bergam P, Di Cicco A, Hurbain I, Lo Cicero A, Dingli F, Palmulli R, Fort C, Potier MC, Schurgers LJ, Loew D, Levy D, Raposo G (2015) Apolipoprotein E regulates amyloid formation within endosomes of pigment cells. Cell Rep 13:43-51.
67. Ebrahimkhani S, Vafaee F, Young PE, Hur SSJ, Hawke S, Devenney E, Beadnall H, Barnett MH, Suter CM, Buckland ME (2017) Exosomal microRNA signatures in multiple sclerosis reflect disease status. Sci Rep 7:14293.
68. Song Y, Hu M, Zhang J, Teng ZQ, Chen C (2019) A novel mechanism of synaptic and cognitive impairments mediated via microRNA-30b in Alzheimer's disease. EBioMedicine 39:409-421.
69. Lugli G, Cohen AM, Bennett DA, Shah RC, Fields CJ, Hernandez AG, Smalheiser NR (2015) Plasma exosomal miRNAs in persons with and without Alzheimer disease: altered expression and prospects for biomarkers. PLoS One 10:e0139233.
70. Cheng L, Doecke JD, Sharples RA, Villemagne VL, Fowler CJ, Rembach A, Martins RN, Rowe CC, Macaulay SL, Masters CL, Hill AF (2015) Prognostic serum miRNA biomarkers associated with Alzheimer's disease shows concordance with neuropsychological and neuroimaging assessment. Mol Psychiatry 20:1188-1196.
71. Shen YF, Zhu ZY, Qian SX, Xu CY, Wang YP (2020) miR-30b protects nigrostriatal dopaminergic neurons from MPP(+)-induced neurotoxicity via SNCA. Brain Behav 10:e01567.
72. Selmaj I, Cichalewska M, Namiecinska M, Galazka G, Horzel-

ski W, Selmaj KW, Mycko MP (2017) Global exosome transcriptome profiling reveals biomarkers for multiple sclerosis. Ann Neurol 81:703-717.
73. Bergman P, Piket E, Khademi M, James T, Brundin L, Olsson T, Piehl F, Jagodic M (2016) Circulating miR-150 in CSF is a novel candidate biomarker for multiple sclerosis. Neurol Neuroimmunol Neuroinflamm 3:e219.
74. Mohammadinasr M, Montazersaheb S, Molavi O, Kahroba H, Talebi M, Ayromlou H, Hejazi MS (2023) Multiplex analysis of cerebrospinal fluid and serum exosomes MicroRNAs of untreated relapsing remitting multiple sclerosis (RRMS) and proposing noninvasive diagnostic biomarkers. Neuromolecular Med 25:402-414.
75. Ahlbrecht J, Martino F, Pul R, Skripuletz T, Sühs KW, Schauerte C, Yildiz Ö, Trebst C, Tasto L, Thum S, Pfanne A, Roesler R, Lauda F, Hecker M, Zettl UK, Tumani H, Thum T, Stangel M (2016) Deregulation of microRNA-181c in cerebrospinal fluid of patients with clinically isolated syndrome is associated with early conversion to relapsing-remitting multiple sclerosis. Mult Scler 22:1202-1214.
76. Gandhi R (2015) miRNA in multiple sclerosis: search for novel biomarkers. Mult Scler 21:1095-1103.
77. Nuzziello N, Blonda M, Licciulli F, Liuni S, Amoruso A, Valletti A, Consiglio A, Avolio C, Liguori M (2017) Molecular characterization of peripheral extracellular vesicles in clinically isolated syndrome: preliminary suggestions from a pilot study. Med Sci (Basel) 5:19.
78. Vistbakka J, Elovaara I, Lehtimäki T, Hagman S (2017) Circulating microRNAs as biomarkers in progressive multiple sclerosis. Mult Scler 23:403-412.
79. Kimura K, Hohjoh H, Fukuoka M, Sato W, Oki S, Tomi C, Yamaguchi H, Kondo T, Takahashi R, Yamamura T (2018) Circulating exosomes suppress the induction of regulatory T cells via let-7i in multiple sclerosis. Nat Commun 9:17.
80. D'Anca M, Fenoglio C, Buccellato FR, Visconte C, Galimberti D, Scarpini E (2021) Extracellular vesicles in multiple sclerosis: role in the pathogenesis and potential usefulness as biomarkers and therapeutic tools. Cells 10:1733.
81. Lee J, McKinney KQ, Pavlopoulos AJ, Han MH, Kim SH, Kim HJ, Hwang S (2016) Exosomal proteome analysis of cerebrospinal fluid detects biosignatures of neuromyelitis optica and multiple sclerosis. Clin Chim Acta 462:118-126.
82. Ma X, Zhou J, Zhong Y, Jiang L, Mu P, Li Y, Singh N, Nagarkatti M, Nagarkatti P (2014) Expression, regulation and function of microRNAs in multiple sclerosis. Int J Med Sci 11:810-818.
83. Winkler A, Wrzos C, Haberl M, Weil MT, Gao M, Möbius W, Odoardi F, Thal DR, Chang M, Opdenakker G, Bennett JL, Nessler S, Stadelmann C (2021) Blood-brain barrier resealing in neuromyelitis optica occurs independently of astrocyte regeneration. J Clin Invest 131:e141694.
84. Ehrengruber MU, Geiser T, Deranleau DA (1994) Activation of human neutrophils by C3a and C5A. Comparison of the effects on shape changes, chemotaxis, secretion, and respiratory burst. FEBS Lett 346:181-184.
85. Chen C, Wu Y, Li M, Cui C, Zhao Y, Sun X, Wang Y, Liu C, Wu H, Zhong X, Kermode AG, Peng L, Qiu W (2020) Different exosomal microRNA profile in aquaporin-4 antibody positive neuromyelitis optica spectrum disorders. Front Immunol 11:1064.
86. Patterson H, Nibbs R, McInnes I, Siebert S (2014) Protein kinase inhibitors in the treatment of inflammatory and autoimmune diseases. Clin Exp Immunol 176:1-10.
87. Mor A, Philips MR, Pillinger MH (2007) The role of Ras signaling in lupus T lymphocytes: biology and pathogenesis. Clin Immunol 125:215-223.
88. Xie Y, Chen B, Wang Q, Chen X, Lai W, Xu Y, Deng S, Yu Z, Xie M, Bu B, Mou D, Yi C, Ding F, Wang W (2023) Astrocyte-derived exosomes contribute to pathologies of neuromyelitis optica spectrum disorder in rodent model. Ann Neurol 94:163-181.
89. Sheremata WA, Jy W, Delgado S, Minagar A, McLarty J, Ahn Y (2006) Interferon-beta1a reduces plasma CD31+ endothelial microparticles (CD31+EMP) in multiple sclerosis. J Neuroinflammation 3:23.
90. Jimenez J, Jy W, Mauro LM, Horstman LL, Ahn ER, Ahn YS, Minagar A (2005) Elevated endothelial microparticle-monocyte complexes induced by multiple sclerosis plasma and the inhibitory effects of interferon-beta 1b on release of endothelial microparticles, formation and transendothelial migration of monocyte-endothelial microparticle complexes. Mult Scler 11:310-315.
91. Manna I, Iaccino E, Dattilo V, Barone S, Vecchio E, Mimmi S, Filippelli E, Demonte G, Polidoro S, Granata A, Scannapieco S, Quinto I, Valentino P, Quattrone A (2018) Exosome-associated miRNA profile as a prognostic tool for therapy response monitoring in multiple sclerosis patients. FASEB J 32:4241-4246.
92. Sáenz-Cuesta M, Alberro A, Muñoz-Culla M, Osorio-Querejeta I, Fernandez-Mercado M, Lopetegui I, Tainta M, Prada Á, Castillo-Triviño T, Falcón-Pérez JM, Olascoaga J, Otaegui D (2018) The first dose of fingolimod affects circulating extracellular vesicles in multiple sclerosis patients. Int J Mol Sci 19:2448.
93. Zinger A, Latham SL, Combes V, Byrne S, Barnett MH, Hawke

S, Grau GE (2016) Plasma levels of endothelial and B-cell-derived microparticles are restored by fingolimod treatment in multiple sclerosis patients. Mult Scler 22:1883-1887.

94. Li Z, Liu F, He X, Yang X, Shan F, Feng J. Exosomes derived from mesenchymal stem cells attenuate inflammation and demyelination of the central nervous system in EAE rats by regulating the polarization of microglia. Int Immunopharmacol. 2019 Feb;67:268-280.
95. Zhang J, Buller BA, Zhang ZG, Zhang Y, Lu M, Rosene DL, Medalla M, Moore TL, Chopp M (2022) Exosomes derived from bone marrow mesenchymal stromal cells promote remyelination and reduce neuroinflammation in the demyelinating central nervous system. Exp Neurol 347:113895.
96. Baharlooi H, Nouraei Z, Azimi M, Moghadasi AN, Tavassolifar MJ, Moradi B, Sahraian MA, Izad M (2021) Umbilical cord mesenchymal stem cells as well as their released exosomes suppress proliferation of activated PBMCs in multiple sclerosis. Scand J Immunol 93:e13013.
97. Vukusic S, Hutchinson M, Hours M, Moreau T, Cortinovis-Tourniaire P, Adeleine P, Confavreux C; Pregnancy In Multiple Sclerosis Group (2004) Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. Brain 127(Pt 6):1353-1360.
98. Martire S, Montarolo F, Spadaro M, Perga S, Sforza ML, Marozio L, Frezet F, Bruno S, Chiabotto G, Deregibus MC, Camussi G, Botta G, Benedetto C, Bertolotto A (2021) A first phenotypic and functional characterization of placental extracellular vesicles from women with multiple sclerosis. Int J Mol Sci 22:2875.
99. Zhang J, Li H, Fan B, Xu W, Zhang X (2020) Extracellular vesicles in normal pregnancy and pregnancy-related diseases. J Cell Mol Med 24:4377-4388.
100. Robinson DP, Klein SL (2012) Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. Horm Behav 62:263-271.
101. Gatson NN, Williams JL, Powell ND, McClain MA, Hennon TR, Robbins PD, Whitacre CC (2011) Induction of pregnancy during established EAE halts progression of CNS autoimmune injury via pregnancy-specific serum factors. J Neuroimmunol 230:105-113.
102. Motallebnezhad M, Taghizadeh S, Aghaie T, Azimi M, Salari AA, Bozorgmehr M, Safari E, Falak R, Jazayeri MH (2022) Placental extract and exosomes derived from pregnant mice attenuate the development of experimental autoimmune encephalomyelitis. Iran J Allergy Asthma Immunol 21:657-669.
103. Soundara Rajan T, Giacoppo S, Diomede F, Bramanti P, Trubiani O, Mazzon E (2017) Human periodontal ligament stem cells secretome from multiple sclerosis patients suppresses NALP3 inflammasome activation in experimental autoimmune encephalomyelitis. Int J Immunopathol Pharmacol 30:238-252.
104. Fan J, Han Y, Sun H, Sun S, Wang Y, Guo R, Guo J, Tian X, Wang J, Wang J (2023) Mesenchymal stem cell-derived exosomal microRNA-367-3p alleviates experimental autoimmune encephalomyelitis via inhibition of microglial ferroptosis by targeting EZH2. Biomed Pharmacother 162:114593.
105. Wang J, Sun H, Guo R, Guo J, Tian X, Wang J, Sun S, Han Y, Wang Y (2023) Exosomal miR-23b-3p from bone mesenchymal stem cells alleviates experimental autoimmune encephalomyelitis by inhibiting microglial pyroptosis. Exp Neurol 363:114374.
106. Wang YK, Zhao YP, Ye MZ, Wang L, Lan TS, Wang Y, Qi ZQ (2023) Chimeric CNS-targeting-peptide engineered exosomes for experimental autoimmune encephalomyelitis therapy. Int Immunopharmacol 124(Pt B):110835.
107. Qin D, Wang C, Li D, Guo S (2024) Exosomal miR-23a-3p derived from human umbilical cord mesenchymal stem cells promotes remyelination in central nervous system demyelinating diseases by targeting Tbr1/Wnt pathway. J Biol Chem 300:105487.
108. Haghmorad D, Khaleghian A, Eslami M, Sadeghnejad A, Tarahomi M, Yousefi B (2023) Bone marrow mesenchymal stem cells to ameliorate experimental autoimmune encephalomyelitis via modifying expression patterns of miRNAs. Mol Biol Rep 50:9971-9984.
109. Bolandi Z, Hashemi SM, Abasi M, Musavi M, Aghamiri S, Miyanmahaleh N, Ghanbarian H (2023) In vitro naive CD4+ T cell differentiation upon treatment with miR-29b-loaded exosomes from mesenchymal stem cells. Mol Biol Rep 50:9037-9046.
110. Angelou CC, Wells AC, Vijayaraghavan J, Dougan CE, Lawlor R, Iverson E, Lazarevic V, Kimura MY, Peyton SR, Minter LM, Osborne BA, Pobezinskaya EL, Pobezinsky LA (2020) Differentiation of pathogenic Th17 cells is negatively regulated by Let-7 MicroRNAs in a mouse model of multiple sclerosis. Front Immunol 10:3125.
111. Jia Z, Liu J, Li B, Yi L, Wu Y, Xing J, Wang L, Wang J, Guo L (2022) Exosomes with FOXP3 from gene-modified dendritic cells ameliorate the development of EAE by regulating the balance of Th/Treg. Int J Med Sci 19:1265-1274.
112. Wu L, Xia J, Li D, Kang Y, Fang W, Huang P (2020) Mechanisms of M2 macrophage-derived exosomal long non-coding RNA PVT1 in regulating Th17 cell response in experimental autoimmune encephalomyelitisa. Front Immunol 11:1934.

113. Baharlooi H, Salehi Z, Minbashi Moeini M, Rezaei N, Azimi M (2022) Immunomodulatory potential of human mesenchymal stem cells and their exosomes on multiple sclerosis. Adv Pharm Bull 12:389-397.
114. Osorio-Querejeta I, Carregal-Romero S, Ayerdi-Izquierdo A, Mäger I, A NL, Wood M, Egimendia A, Betanzos M, Alberro A, Iparraguirre L, Moles L, Llarena I, Möller M, Goñi-de-Cerio F, Bijelic G, Ramos-Cabrer P, Muñoz-Culla M, Otaegui D (2020) MiR-219a-5p enriched extracellular vesicles induce OPC differentiation and EAE improvement more efficiently than liposomes and polymeric nanoparticles. Pharmaceutics 12:186.
115. Ruckh JM, Zhao JW, Shadrach JL, van Wijngaarden P, Rao TN, Wagers AJ, Franklin RJ (2012) Rejuvenation of regeneration in the aging central nervous system. Cell Stem Cell 10:96-103.
116. Pusic AD, Pusic KM, Clayton BL, Kraig RP (2014) IFNγ-stimulated dendritic cell exosomes as a potential therapeutic for remyelination. J Neuroimmunol 266:12-23.
117. Pusic AD, Kraig RP (2014) Youth and environmental enrichment generate serum exosomes containing miR-219 that promote CNS myelination. Glia 62:284-299.