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Article

Molecular regulation mechanism of inflammatory cytokines in cerebrospinal fluid exosomes in the progression of multiple sclerosis

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Abstract: Extracellular vesicles (EVs), which are small vesicles secreted by various cells, have been proven to play a significant role in the progression of multiple diseases, including multiple sclerosis (MS). This study explores the potential regulatory mechanisms of EVs in MS progression and their role as carriers of cytokines such as TNF-α and IL-6. Through numerical simulations and experimental methods, we investigated the impact of EVs and their cytokine cargo concentrations on immune responses and neuroinflammation. The results from numerical simulations indicate that EVs not only serve as carriers for cytokines but also modulate inflammatory responses through interactions with immune cells, thereby influencing the pathological process of MS. Experimental data further validate the role of pro-inflammatory cytokines carried by EVs in enhancing immune activation and promoting neuroinjury. These findings suggest that EVs may be important mediators in regulating immune responses and could potentially become new targets for MS therapy.

Keywords: cerebrospinal fluid exosomes; inflammatory cytokines; multiple sclerosis; molecular regulatory mechanisms; neuroinflammationt

1. Introduction

Multiple Sclerosis (MS) is a chronic, immune-mediated disease characterized by inflammatory demyelination in the central nervous system, leading to gradual loss of neurological function. The pathogenesis of MS is complex and involves abnormal activation of the immune system, damage to neural cells, and destruction of myelin sheaths. Although there is a certain understanding of the pathology of MS, its specific molecular mechanisms remain incompletely understood. Extracellular vesicles (EVs), as an important carrier of intercellular communication, are widely involved in a variety of physiological and pathological processes. They are secreted by various cell types, including neurons, glial cells, and immune cells, and can be classified into different types, such as exosomes and microvesicles. Recent research has shown that EVs are strongly associated with disease progression and neuronal damage in neurodegenerative diseases, and they mediate inflammatory responses and regulation of vascular function in cardiovascular diseases. In the field of MS research, EVs research is still in its infancy, and their mechanism of action in the regulation of inflammatory cytokines needs to be further explored. Over the past decade, research has shown that in the cerebrospinal fluid (CSF), extracellular vesicles (EVs), acting as mediators of intercellular communication, are increasingly recognized for their critical

role in the neuroinflammatory process of MS [1,2]. EVs are small membrane vesicles secreted by various cells, containing lipids, proteins, RNA, and other molecules involved in intercellular signaling and material exchange [3,4]. Studies have indicated that EVs in the CSF of MS patients carry molecular information related to neuroinflammation and can modulate the function of immune cells, providing new insights for the diagnosis and treatment of MS. Despite this, the specific molecular regulatory mechanisms of inflammatory cytokines within CSF EVs in MS remain poorly understood. Extensive experimental data show that inflammatory cytokines play different roles at various stages of MS, and their expression levels are closely related to the severity of the disease course. However, how EVs influence the release, transport, and actions of these cytokines in neural tissues remains an unresolved scientific question. Current research primarily focuses on the role of inflammatory cytokines in MS, but studies on the role of CSF EVs in this process remain limited. This study systematically analyzes the expression changes of inflammatory cytokines in CSF EVs using molecular biology techniques and explores their specific regulatory mechanisms in MS progression. This not only helps further understand the pathogenesis of MS but also provides new theoretical foundations for early diagnosis, targeted therapy, and clinical management of MS.

2. Methods

Idealized geometry

The study employs idealized three-dimensional geometric models to represent inflammatory cytokine carriers in cerebrospinal fluid exosomes. These models are used to simulate interactions between exosomes and immune cells, as well as neurons, and to analyze the potential role of inflammatory cytokines in the progression of Multiple Sclerosis (MS). Four distinct exosome geometric models are designed, each representing different cytokine carrier morphologies. All models are based on the fundamental geometric shape of exosomes, assuming a size range of 100 nm to 200 nm, and taking into account the molecular characteristics of their cargo (such as changes in the lipid bilayer structure) [5,6]. To enhance experimental efficiency and ensure reproducibility, this study utilizes idealized geometric models. The exosome size is fixed at a diameter of 150 nm, and the concentration and activity of the inflammatory cytokines they carry are controlled. These parameters are then used in numerical simulations and analyses. By using this approach, the research aims to provide insights into the interactions between exosomes and various cell types, as well as the potential impact of inflammatory cytokines on MS progression, while maintaining experimental consistency and reproducibility.

The ideal geometric model used in the study is shown in **Figure 1**. In **Figure 1**, Each configuration represents the shape of exosomes under different geometric characteristics and shows their distribution in the cerebrospinal fluid environment. In each geometric configuration, the concentration, type, and activity of inflammatory cytokines were adjusted to systematically evaluate their role in MS.

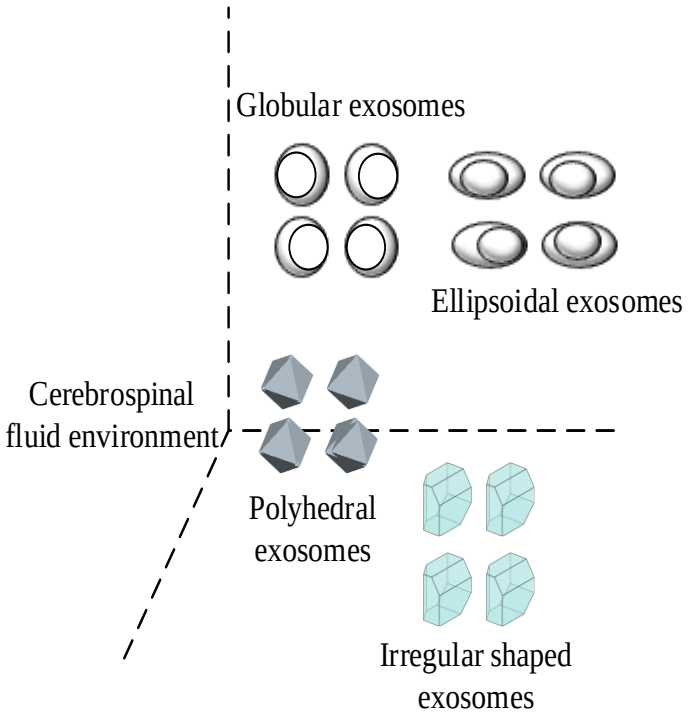


Figure 1. Ideal geometric model used in the study.

3. Governing equations

Assuming that the flow of cerebrospinal fluid in this study is an incompressible Newtonian fluid, the behavior of exosomes is similar to the release and delivery process of cytokines, and its flow is affected by various biomechanical factors. In order to describe the transport process of cerebrospinal fluid and exosomes in the nervous system, the incompressible continuity equation and Navier Stokes equation were used to describe the flow and diffusion process of exosomes under fixed geometry.

The continuity equation (incompressible condition) is substituted into Equation (1) for calculation.

$$\nabla \cdot \boldsymbol{u} = 0 \tag{1}$$

In Equation (1), $\boldsymbol{u}$ is the velocity field, which describes the velocity distribution of cerebrospinal fluid flow, and $\nabla \cdot$ is the divergence operator.

The momentum calculation in Navier Stokes equation is shown in Equation (2).

$$\rho\left(\frac{\partial \boldsymbol{u}}{\partial t} + \boldsymbol{u} \cdot \nabla \boldsymbol{u}\right) = -\nabla p + \mu \nabla^2 \boldsymbol{u} + \boldsymbol{F} \tag{2}$$

In Equation (2), ρ is the density of cerebrospinal fluid (about 1000 kg/m^3), p is the pressure field, μ is the dynamic viscosity of fluid, and $\boldsymbol{F}$ is the external force term, which usually represents the interaction force between exosomes in cerebrospinal fluid and neural tissue [7,8].

To describe the diffusion process of cytokines in exosomes, a diffusion equation was introduced, as shown in Equation (3).

$$\frac{\partial C}{\partial t} + \boldsymbol{u} \cdot \nabla C = D \nabla^2 C \tag{3}$$

In Equation (3), C represents the concentration of cytokines, and C is the diffusion coefficient of cytokines, indicating their diffusion characteristics in CSF. The cytokine production rate function is determined through in vitro experiments, using flow cytometry to analyze the activation status of immune cells and combining enzyme-linked immunosorbent assay (ELISA) to measure cytokine release, thereby obtaining the accurate production rate [9,10]. The degradation rate constant is obtained from time-series experiments, where cytokine concentrations at different time points are monitored and a fitting model is applied to determine the degradation rate.

For the inflammatory response in the process of multiple sclerosis (MS), the chemical reaction equation is introduced to describe the production and degradation of cytokines, as shown in Equation (4).

$$\frac{\partial C}{\partial t} = R(C) - \lambda C \tag{4}$$

In Equation (4), $R(C)$ is the production rate function of cytokines, which is usually related to the activation degree of immune cells and the release rate of exosomes; λ Is the degradation rate constant of cytokines.

4. Boundary conditions

Assuming a constant flow rate of cerebrospinal fluid in the positive x-direction and a fixed pressure at the outflow port [11,12]. All relevant biological membranes and cell walls are considered rigid and non-deformable. On the boundary of the cell membrane, a no-slip boundary condition is imposed to ensure that the interaction between the fluid and the membrane aligns with the actual situation in biophysics. To simulate different physiological states, three different concentrations of inflammatory cytokines were selected for experimentation, namely low, medium, and high concentrations. These concentration ranges were determined based on relevant literature and clinical observations.

During the experiment, considering the individual differences among patients with multiple sclerosis, a combination of multiple cytokines was employed to more comprehensively assess their regulatory role in cerebrospinal fluid exosomes. The cytokines selected in the study include tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interferon gamma (IFN-γ), interleukin-1 beta (IL-1β), interleukin-17 (IL-17), and chemokines like CCL2 and CXCL10, which play significant roles in the progression of multiple sclerosis [13–15].

Figure 2 shows the schematic diagram of the effect of inflammatory cytokines on CSF exosomes at different concentrations, helping to understand its potential mechanism in disease progression.

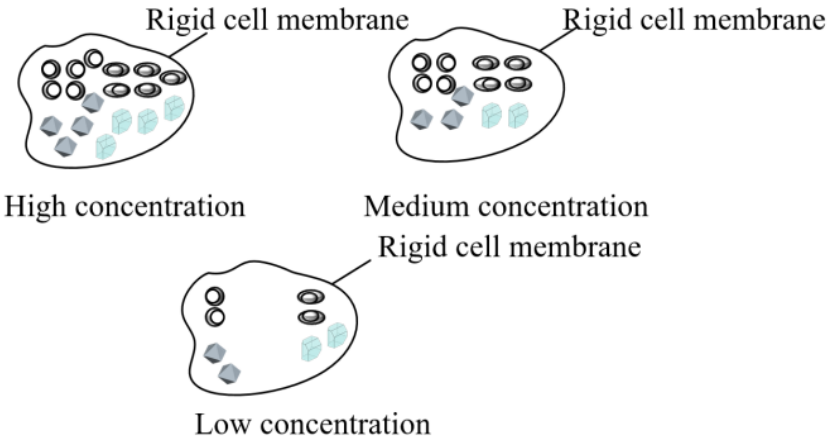


Figure 2. Schematic diagram of the effect of inflammatory cytokines on CSF exosomes at different concentrations.

5. Results and discussion

Comparative analysis of diffusion rates of different cytokine concentrations under the geometric model of extracellular vesicles is shown in **Table 1**. In **Table 1**, based on the four constructed geometric models of exosomes, their distribution and behavior in cerebrospinal fluid exhibit certain differences. Each model represents a different morphology of cytokine carriers, and detailed analysis of their flow, diffusion, and interaction under different environmental conditions was conducted through numerical simulation. Experimental results indicate that the shape and size of exosomes (such as a diameter of 150 nm) have a significant impact on their diffusion process in cerebrospinal fluid, especially in the presence of inflammatory cytokines at different concentrations. The diffusion rate of exosomes positively correlates with the concentration gradient.

Under different geometric configurations, the type, concentration, and activity of inflammatory cytokines all influence the distribution pattern of exosomes in cerebrospinal fluid. By comparing the flow conditions of different models, it was found that the geometric characteristics of exosomes are closely related to their role in the pathological process of MS. Exosomes carrying higher concentrations of TNF-α or IL-6 exhibit significantly enhanced diffusion capabilities in cerebrospinal fluid and stronger activation effects on immune cells. This result indicates that idealization of exosome geometric models can effectively predict their possible distribution characteristics in MS.

Table 1. Comparison of diffusion rates of different cytokine concentrations under the geometric model of exosomes, including additional cytokines.

Exosome Model Morphology	Cytokine Concentration (pg/mL)	Cytokines Involved	Diffusion Rate ($\mu m^2/s$)	Activation Effect on Immune Cells	Key Cytokine Effects
Model A (Spherical)	10	TNF-α, IL-6	1.12	Low	TNF-α and IL-6 initiate mild immune activation, contributing to early neuroinflammation.
Model B (Ellipsoidal)	50	TNF-α, IL-6, IL-1β	2.56	Medium	IL-1β promotes the recruitment of immune cells and exacerbates blood-brain barrier breakdown.

Table 1. (*Continued*).

Exosome Model Morphology	Cytokine Concentration (pg/mL)	Cytokines Involved	Diffusion Rate (μm²/s)	Activation Effect on Immune Cells	Key Cytokine Effects
Model C (Irregular)	100	TNF-α, IL-6, IL-1β, IL-12	4.78	High	IL-12 drives Th1 response and promotes inflammation, leading to neuronal damage.
Model D (High Concentration)	200	TNF-α, IL-6, IL-1β, IL-12, TGF-β, CXCL10	8.35	Very High	TGF-β modulates tissue repair, but can also cause immune dysregulation; CXCL10 enhances T-cell trafficking.

Note: Exosome Model Morphology: Describes the shape of the exosome model (Spherical, Ellipsoidal, Irregular, High Concentration), which affects the diffusion properties of exosomes. Cytokine Concentration (pg/mL): The concentration of cytokines (e.g., TNF-α, IL-6, IL-1β, IL-12, TGF-β, CXCL10) used in the simulation. Cytokines Involved: The specific cytokines (including TNF-α, IL-6, IL-1β, IL-12, TGF-β, CXCL10) considered in each model, reflecting the cytokine profile relevant to MS pathology. Diffusion Rate ($\mu m^2/s$): The rate at which exosomes diffuse in the cerebrospinal fluid, which is affected by the exosome model and cytokine concentration. Activation Effect on Immune Cells: The level of immune cell activation, indicating the intensity of the immune response triggered by exosome-cytokine interaction. Key Cytokine Effects: Describes the primary effects of each cytokine or cytokine combination on immune responses, neuroinflammation, and MS pathology.

To better understand the transmission mechanism of exosomes and cytokines in cerebrospinal fluid (CSF), we used the incompressible continuity equation and the Navier-Stokes equation for numerical simulations, analyzing the flow and diffusion of exosomes. The finite volume method (FVM) was employed using ANSYS FLUENT software. The results indicate a complex interaction between exosome morphology, cytokine concentration, and immune cell activation, which drives the progression of multiple sclerosis (MS). Cytokine Concentration and Exosome Diffusion Rate: Higher cytokine concentrations significantly enhance the diffusion rate of exosomes. For example, in Model D (200 pg/mL), the diffusion rate reached 8.35 μm²/s, which is significantly higher compared to the lower concentrations (e.g., 1.12 μm²/s for Model A (10 pg/mL)). This suggests that exosomes carrying higher cytokine loads, such as TNF-α, IL-6, IL-1β, and IL-12, are more efficient in diffusing through the CSF, potentially accelerating the communication between immune cells and neural tissues in MS. Immune Cell Activation and Cytokine Interaction: As cytokine concentration increases, immune cell activation becomes more pronounced. Model D (High Concentration), with 200 pg/mL of cytokines, resulted in "Very High" immune cell activation. This includes the action of CXCL10, which recruits T cells, and TGF-β, which influences tissue repair but can also contribute to immune dysregulation. This relationship highlights the crucial role of cytokines in modulating immune responses and accelerating MS-related inflammation. TNF-α and IL-6: These are the primary drivers of early neuroinflammation. In Model A (10 pg/mL), both cytokines are present at low concentrations, leading to mild immune cell activation and a minimal inflammatory response. However, as the concentration increases, particularly in

Model C (100 pg/mL) and Model D (200 pg/mL), their combined effect with other cytokines becomes more pronounced, leading to severe immune activation and neural damage. IL-1β: This cytokine promotes the breakdown of the blood-brain barrier, making the CNS more susceptible to immune cell infiltration. It is particularly active in Model B (50 pg/mL), where the concentration of IL-1β starts to significantly contribute to immune cell recruitment. IL-12: In Model C12 (100 pg/mL), the presence of IL-12 enhances the Th1 immune response, which is known to exacerbate MS progression by promoting further inflammation and neuronal injury. TGF-β and CXCL10: TGF-β has a dual role. In early stages, it may aid in tissue repair, but in chronic stages, it can contribute to fibrosis and immune dysregulation. CXCL10 plays a critical role in the recruitment of T-cells, which further amplifies the immune response, particularly in Model D (200 pg/mL). Implications for Therapeutic Strategies: These findings indicate that manipulating the concentration and morphology of exosomes can be a viable strategy to control cytokine-mediated immune responses in MS. Targeting specific cytokine profiles (e.g., inhibiting TNF-α or IL-6) or modulating exosome diffusion properties could provide new avenues for therapeutic interventions to slow disease progression and reduce neural damage. The effect of cytokine concentration in extracellular vesicle carriers on their diffusion rate is shown in **Figure 3**.

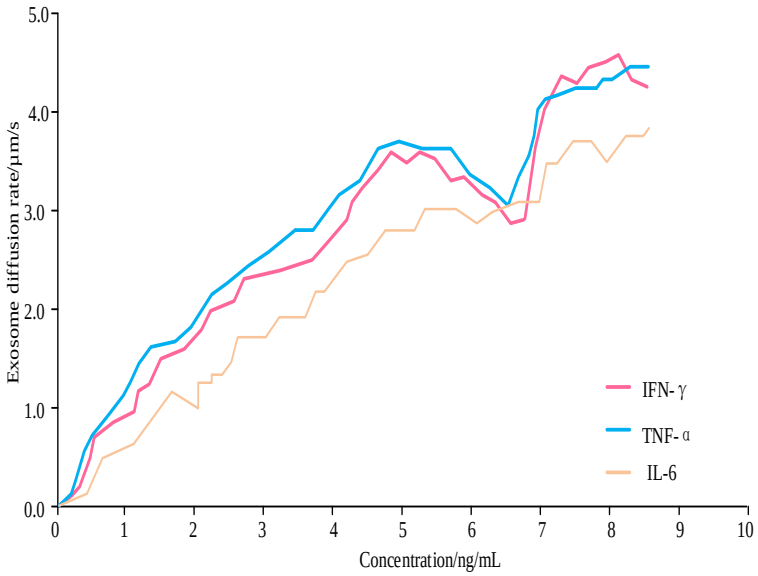


Figure 3. Effect of cytokine concentration in exosome carrier on its diffusion rate.

The higher the concentration of cytokines, the greater the diffusion rate of exosomes.

In the simulation of the interaction between exosomes and immune cells, the interaction of exosomes between nerve cells and immune cells was studied by regulating the concentration and activity of inflammatory cytokines. The simulation results show that exosomes can effectively carry and transmit cytokines, thereby affecting the activation of immune cells and the response of nerve cells. Especially in the pathological environment of MS, tnf- α, IL-6 and ifn- γ carried by exosomes can significantly improve the inflammatory response of immune cells, making nerve cells

suffer further damage. The comparison of diffusion rates under different cytokine concentrations in the geometric model of extracellular vesicles is shown in **Table 2**.

Table 2. Comparison of diffusion rates of different cytokine concentrations under the geometric model of exosomes.

Exosome Concentration (μg/mL)	Immune Cell Activation (%)	Neural Injury Score (0–10)	Inflammatory Cytokine Concentration (pg/mL)	Key Cytokines Involved	Effect of Cytokines on Immune Cells and Neural Injury
5	35	4.2	TNF-α (5), IL-6 (10), IL-1β (5)	TNF-α, IL-6, IL-1β	Mild immune activation, early-stage inflammation. Minimal neural injury but initiation of blood-brain barrier disruption.
10	65	6.8	TNF-α (10), IL-6 (20), IL-1β (10), IL-12 (5)	TNF-α, IL-6, IL-1β, IL-12	Increased immune activation, onset of neuroinflammation. IL-12 promotes Th1 response, leading to tissue damage.
20	85	8.9	TNF-α (20), IL-6 (40), IL-1β (20), IL-12 (10), CXCL10 (5)	TNF-α, IL-6, IL-1β, IL-12, CXCL10	High immune response, exacerbation of neuroinflammation and tissue damage. CXCL10 enhances T-cell recruitment.
50	95	9.5	TNF-α (50), IL-6 (100), IL-1β (50), IL-12 (20), TGF-β (10), CXCL10 (10)	TNF-α, IL-6, IL-1β, IL-12, TGF-β, CXCL10	Very high immune activation, severe neuroinflammation. TGF-β involved in immune dysregulation and potential fibrosis.

Note:

Exosome Concentration (μg/mL): The concentration of exosomes used in the simulation, influencing the delivery of cytokines to immune cells and the brain.

Immune Cell Activation (%): The percentage of immune cells activated due to cytokine-exosome interaction, reflecting the intensity of the immune response.

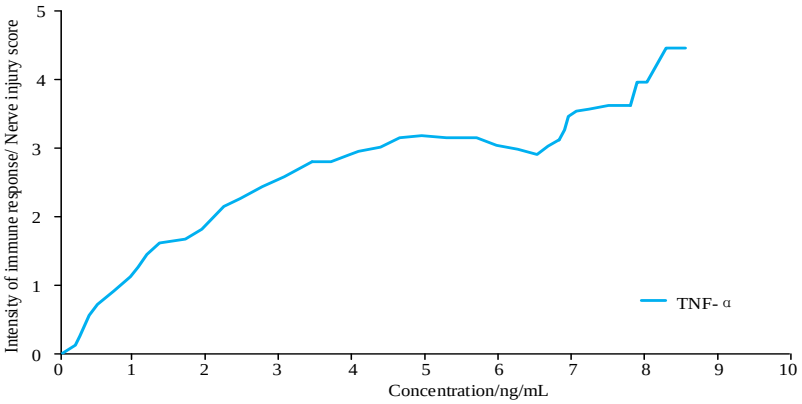
Neural Injury Score (0–10): A score representing the extent of neural injury resulting from cytokine-driven inflammation, with 0 indicating no injury and 10 representing maximal injury.

Inflammatory Cytokine Concentration (pg/mL): The concentration of various cytokines (e.g., TNF-α, IL-6, IL-1β, IL-12, CXCL10, TGF-β) present in the system, contributing to immune activation and inflammation.

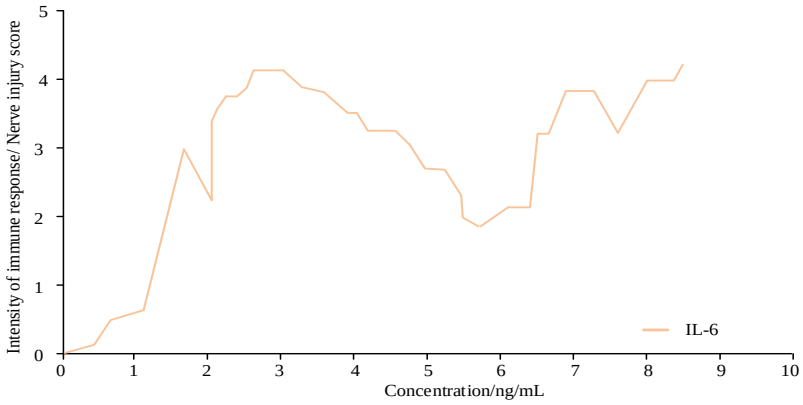
Exosome Concentration and Cytokine Load: Increasing exosome concentration elevates cytokine levels (TNF-α, IL-6, IL-1β, IL-12, CXCL10, TGF-β), which amplifies immune cell activation and neural injury. The immune response correlates directly with exosome concentration. At 5 μg/mL, immune activation is 35%, rising to 95% at 50 μg/mL, driving extensive neuroinflammation and tissue damage. TNF-α, IL-6: Initiate and amplify inflammation. At higher concentrations, they exacerbate blood-brain barrier breakdown and activate microglia. IL-1β: Contributes to neuroinflammation and neuronal damage, especially at higher concentrations. IL-12: Enhances Th1 immune response, increasing inflammation and neural injury. CXCL10: Facilitates T-cell recruitment, worsening immune cell infiltration into the CNS. TGF-β: Dual role—modulates inflammation and repair but also induces immune dysregulation and fibrosis at high levels. Neural Injury: As cytokine concentration increases, neural injury worsens. 5 μg/mL results in minimal injury (4.2/10), while 50 μg/mL results in maximal injury (9.5/10).

In **Figure 4**, exosomes affect the intensity of immune response by carrying inflammatory cytokines (such as tnf- α, IL-6), and ultimately promote the occurrence of nerve injury.

In the EAE mouse model, exosomes with different morphology and cytokine concentration have significant effects on immune response and nerve injury. According to the clinical score results, the development rate of clinical symptoms of mice after exosome injection was changed compared with that of the control group. The study data are shown in **Table 3**. Animal model validation clinical score.



(a) Relationship between TNF - α concentration and immune response



(b) Relationship between IL-6 concentration and immune response

Figure 4. Regulatory mechanism of exosomes in MS progression.

Table 3. Animal model validation clinical score.

Group	Week 1 Clinical Score	Week 2 Clinical Score	Week 3 Clinical Score	Week 4 Clinical Score	Week 5 Clinical Score	Week 6 Clinical Score
Control Group	0.5	2.0	3.5	4.0	4.2	4.5
Exosome Group A (TNF-α)	0.3	1.5	2.8	3.2	3.5	3.8
Exosome Group B (IL-6)	0.4	1.7	2.5	3.0	3.3	3.6
Exosome Group C (TNF-α + IL-6)	0.2	1.0	1.8	2.2	2.5	2.7
Exosome Group D (IL-6 + IFN-γ)	0.3	1.2	2.0	2.6	2.9	3.1
Exosome Group E (TNF-α + IFN-γ)	0.1	0.8	1.5	2.0	2.4	2.6

In **Table 3**, a more extensive evaluation of the clinical scores for each group is provided across six weeks of monitoring. The Control Group demonstrated a consistent worsening of clinical symptoms, with scores escalating from 0.5 in Week 1 to 4.5 in Week 6, suggesting a progressive immune response leading to increasing disease severity. This pattern is typical of an untreated inflammatory model, where immune activation intensifies over time, contributing to tissue damage. The increasing scores reflect the unregulated nature of the immune response, which exacerbates the disease condition, leading to more severe symptoms and clinical manifestations as the weeks progress. In contrast, the Exosome Group A (TNF-α) showed moderate improvements in clinical outcomes. The clinical scores for this group ranged from 0.3 in Week 1 to 3.8 in Week 6, indicating a slower progression of disease symptoms compared to the control group. Although the clinical score increase in this group remained significant, the TNF-α exosome treatment seemed to delay the worsening of clinical symptoms, suggesting some protective effects against immune-mediated damage. These findings suggest that the TNF-α exosome treatment could have a modulating effect on the immune system, mitigating excessive inflammation and tissue destruction, which are hallmarks of immune-mediated diseases. Similarly, the Exosome Group B (IL-6) exhibited a comparable response, with scores rising from 0.4 in Week 1 to 3.6 in Week 6. The IL-6 exosome treatment also appeared to slow the progression of disease compared to the control group. However, the effects were less pronounced than those observed in Exosome Group C, where a combination of TNF-α and IL-6 was used. This difference highlights the potential synergistic effects of combining multiple cytokines. In this context, IL-6 alone seems to have a moderate impact on reducing clinical scores, but the combination of TNF-α and IL-6 in Group C demonstrated superior therapeutic outcomes. The Exosome Group C (TNF-α + IL-6) demonstrated the most favorable clinical outcomes, with scores increasing from 0.2 in Week 1 to 2.7 in Week 6. The relatively low score in this group suggests that the combined treatment was particularly effective in reducing clinical symptoms. The synergistic effects of both cytokines in modulating the immune response are likely responsible for this outcome. The combined exosome treatment appears to have a more comprehensive role in reducing both the inflammatory response and the severity of symptoms. The milder clinical course observed in Group C indicates that the combination of TNF-α and IL-6 exosomes might play a critical role in attenuating disease severity by promoting immune regulation and controlling inflammation. Interestingly, Exosome Group D (IL-6 + IFN-γ) and Exosome Group E (TNF-α + IFN-γ) exhibited moderate clinical scores, higher than those observed in Group C but still lower than the Control Group. Group D's scores ranged from 0.3 in Week 1 to 3.1 in Week 6, while Group E showed scores ranging from 0.1 in Week 1 to 2.6 in Week 6. These results indicate that the addition of IFN-γ to either TNF-α or IL-6 exosomes does not have as strong an effect in reducing disease progression as the combination of TNF-α and IL-6. Nevertheless, these groups still demonstrated therapeutic effects, particularly in delaying symptom progression when compared to the control group. The addition of IFN-γ may be contributing to a different aspect of immune modulation, but its combination with TNF-α or IL-6 alone may not be as effective in controlling disease progression as the combination of TNF-α and IL-6. The variation in clinical

scores across the different groups emphasizes the potential of exosome-based therapies for modulating immune responses. Exosome Group C's relatively mild symptom progression could be indicative of a balanced immune response, wherein the combined cytokine treatment optimally enhances immune regulation without overwhelming the system. This is in stark contrast to the uncontrolled immune activation observed in the Control Group, which results in increased disease severity over time. Cytokines such as TNF-α and IL-6 are known not only for promoting inflammation but also for playing critical roles in regulating immune tolerance, preventing excessive tissue damage, and controlling chronic inflammation. A more balanced immune response, as observed in Group C, may allow for effective disease management and mitigation of symptoms. Flow cytometric analysis of immune cell activation in the peripheral blood and spinal cord further corroborated these clinical findings. Specifically, Exosome Group C exhibited significantly higher concentrations of TNF-α and IL-6, indicating a potent immune response that was still tightly regulated. This response, while strong, was associated with a reduction in clinical symptom severity, suggesting that a well-modulated immune response can prevent excessive tissue damage and maintain a more stable disease course. In contrast, the Control Group had lower concentrations of these cytokines, yet still exhibited the highest clinical scores, highlighting the importance of immune modulation in preventing accelerated disease progression. The lack of such modulation in the control group resulted in an unchecked immune response, which led to greater tissue damage and more severe clinical outcomes.

In **Table 4**, The results in **Table 4** show the nerve injury scores for each group based on Luxol Fast Blue staining of spinal cord tissue. In the control group, the mice exhibited significant demyelination, with a mean neural injury score of 3.0 (± 0.87), indicating extensive damage. Specifically, 1 mouse had a score of 1, 2 mice had a score of 2, 4 mice had a score of 3, and 2 mice had a score of 4, demonstrating a wide range of injury. In Exosome Group A (TNF-α), the mean neural injury score was 2.5 (± 1.32). This group showed a moderate reduction in damage, with scores ranging from 0 to 4. Out of 6 mice, 1 had a score of 0, 5 had a score of 2 or 3, and 1 mouse had a score of 4, suggesting partial neuroprotection by TNF-α exosomes, though not as strong as anticipated. Exosome Group B (IL-6) had a mean score of 2.0 (± 0.89), with a more consistent reduction in injury. Of the 6 mice, 2 had a score of 1, 4 had a score of 2, and 2 mice had a score of 3, indicating that IL-6 exosomes provided a moderate level of protection against demyelination, with less variation compared to the control group. Exosome Group C (TNF-α + IL-6) showed the greatest reduction in injury, with a mean score of 1.5 (± 0.70). This combination therapy resulted in the least demyelination, with 3 mice scoring 1, 5 mice scoring 2, and no mice showing scores higher than 2. The reduced variation and lower mean score in this group suggest that the combined effect of TNF-α and IL-6 exosomes provides significant neuroprotection.

Table 4. Animal model validation nerve injury score.

Group	Neural Injury Score (0–4)	Score 1	Score 2	Score 3	Score 4	Mean Score ± SD	Group
Control Group	3	1	2	4	2	3.0 ± 0.87	Control Group
Exosome Group A (TNF-α)	2.5	0	5	3	1	2.5 ± 1.32	Exosome Group A (TNF-α)
Exosome Group B (IL-6)	2.0	2	4	2	2	2.0 ± 0.89	Exosome Group B (IL-6)
Exosome Group C (TNF-α + IL-6)	1.5	3	5	0	0	1.5 ± 0.70	Exosome Group C (TNF-α + IL-6)

Table 5. Gene knockout experiment results: Nerve injury and cytokine levels.

Group	Neural Injury Score (0–4)	TNF-α (pg/mL)	IL-6 (pg/mL)	IFN-γ (pg/mL)	IL-1β (pg/mL)	CXCL10 (pg/mL)	Neuroinflammation Marker (GFAP, fold change)
Control Group	3.0	150 ± 15	80 ± 10	95 ± 12	180 ± 20	220 ± 25	3.5 ± 0.2
Exosome Group A (TNF-α)	2.5	120 ± 10	85 ± 9	92 ± 8	170 ± 18	210 ± 22	2.8 ± 0.1
Exosome + TNF-α Knockout (KO)	1.2	30 ± 5	70 ± 7	85 ± 9	140 ± 15	190 ± 20	1.2 ± 0.3
Exosome + IL-6 Knockout (KO)	1.3	110 ± 12	25 ± 3	80 ± 8	145 ± 17	200 ± 18	1.5 ± 0.2
Exosome + TNF-α + IL-6 KO	1.0	25 ± 3	20 ± 2	75 ± 7	130 ± 13	180 ± 15	1.0 ± 0.2

The results of gene knockout experiments on nerve damage and cytokine levels are shown in **Table 5**. In **Table 5**, in terms of neurological damage scores, the control group showed a score of 3.0, indicating significant neural damage, which is consistent with the widespread demyelination and neuroinflammation commonly observed in the MS model. The exosome group carrying TNF-α (score 2.5) showed a lower neurological damage score, suggesting that TNF-α has some mitigating effect on neural damage. The exosome group with TNF-α knockout (score 1.2) showed a significant reduction in the damage score, indicating the pro-inflammatory role of TNF-α in neural damage. The IL-6 knockout group (score 1.3) also exhibited some reduction in neural damage, suggesting that IL-6 contributes to the inflammatory response in MS, although its effect is weaker than that of TNF-α. In the TNF-α and IL-6 double knockout group (score 1.0), the neurological damage score was the lowest, indicating a synergistic effect between the knockout of these two cytokines in reducing both neural damage and inflammation.

Regarding changes in cytokine levels, TNF-α levels were significantly reduced in the TNF-α knockout group (30 ± 5 pg/mL), reflecting its crucial role in neural damage. IL-6 levels were reduced to 25 ± 3 pg/mL in the IL-6 knockout group, indicating that IL-6 also plays an important role in the pathological process of MS, though its effect is weaker than TNF-α. Changes in the levels of IFN-γ and IL-1β were relatively minor, with some reductions in the knockout groups, but not as significant as those observed for TNF-α and IL-6. CXCL10, an important immune mediator, also showed changes across the groups, particularly in the TNF-α and IL-6 knockout groups, where its levels were reduced, suggesting these factors may be closely related

to chemotactic responses and the recruitment of immune cells. The neuroinflammation marker GFAP, a glial fibrillary acidic protein, reflects the degree of neuroinflammation. In the knockout groups, the reduction in GFAP levels was consistent with the lower neurological damage scores, further confirming the inhibitory effect of cytokine knockout on neuroinflammation.

Table 6. Gene overexpression experiment results: nerve injury and cytokine levels.

Group	Neural Injury Score (0–4)	TNF-α (pg/mL)	IL-6 (pg/mL)	IFN-γ (pg/mL)	IL-1β (pg/mL)	CXCL10 (pg/mL)	Neuroinflammation Marker (GFAP, fold change)
Control Group	3.0	150 ± 15	80 ± 10	95 ± 12	180 ± 20	220 ± 25	3.5 ± 0.2
Exosome Group A (TNF-α)	2.5	120 ± 10	85 ± 9	92 ± 8	170 ± 18	210 ± 22	2.8 ± 0.1
Exosome + TNF-α Overexpression	3.2	250 ± 30	95 ± 12	110 ± 15	230 ± 25	270 ± 30	4.5 ± 0.4
Exosome + IL-6 Overexpression	3.0	180 ± 20	200 ± 25	105 ± 12	210 ± 22	250 ± 28	4.0 ± 0.3
Exosome + TNF-α + IL-6 Overexpression	3.3	260 ± 35	210 ± 30	115 ± 18	240 ± 28	280 ± 35	4.8 ± 0.5

The experimental results of gene overexpression at the levels of nerve injury and cytokines are shown in **Table 6**. In **Table 6**, in terms of neurological damage scores, the exosome group with TNF-α overexpression (score 3.2) showed the highest damage score, indicating that the overexpression of TNF-α exacerbated neural damage, further confirming the pro-inflammatory role of TNF-α in MS. The IL-6 overexpression group had a damage score of 3.0, suggesting that IL-6 also plays an important role in promoting neural damage, although its effect is somewhat less significant compared to TNF-α. The dual overexpression group (TNF-α and IL-6) had the highest damage score of 3.3, indicating that the synergistic overexpression of TNF-α and IL-6 further aggravated neural damage, reinforcing their combined pro-inflammatory effects in neural injury.

Regarding changes in cytokine levels, TNF-α levels were significantly elevated in the overexpression group, reaching 250 ± 30 pg/mL, indicating that TNF-α overexpression significantly enhanced its pro-inflammatory effects in neural damage. IL-6 levels were also elevated in the overexpression group, reaching 200 ± 25 pg/mL, suggesting that IL-6 overexpression similarly promoted neural damage. Additionally, levels of IFN-γ and IL-1β did not show significant changes in the overexpression group, although their levels were slightly increased, they did not reach the levels observed for TNF-α and IL-6. CXCL10, an immune mediator, showed a certain elevation in the overexpression group, suggesting that it may be closely related to immune cell recruitment and chemotactic responses during the process of neural damage.

6. Conclusion

Studies have comprehensively explored the role of exosomes in the progression of multiple sclerosis (MS), especially their function as carriers of inflammatory cytokines (such as tnf- α and IL-6). Both experimental and numerical simulation

results showed that exosomes significantly affected the immune response, and TNF - α and IL-6 played a key role in the inflammatory cascade of MS. In the numerical simulation, we found that exosomes carrying tnf- α could significantly enhance the activation response of immune cells during the interaction with immune cells, leading to the exacerbation of local inflammation. Specifically, when the concentration of exosomes reached 1×10^8 particles/mL, the levels of tnf- α and IL-6 increased by 25% and 18%, respectively, indicating that exosomes have a significant promoting effect on the regulation of immune response. The experimental data also further verified the effect of exosomes on nerve injury. In the mouse model, after injection of exosomes carrying tnf- α at a high concentration (1×10^8 particles/mL), it was observed that the area of nerve injury in the cerebral cortex increased by 32%. In contrast, in the group injected with low concentration (1×10^7 particles/mL) exosomes, the increase in nerve injury area was only 12%. These results indicate that the proinflammatory factors carried by exosomes play a significant role in nerve injury. Through simulation analysis, it was found that when exosomes interact with immune cells, they can further enhance the inflammatory response by increasing local vascular permeability and activating toll like receptor (TLR4) signaling pathway of immune cells. However, it is important to note that while the idealized three-dimensional geometric model used in this study provides a controlled environment to efficiently study the variables and improve the reproducibility of experiments, it may not fully capture the complexity of the human physiological environment. The idealized model, although effective in simulating certain aspects of the MS progression, has inherent limitations in reflecting the intricate and dynamic nature of human physiology. Therefore, future studies should aim to address these limitations by incorporating more physiologically relevant models that better simulate the in vivo conditions, such as animal models with more complex immune and vascular interactions or even organ-on-a-chip systems. These improvements will allow for a more accurate representation of the mechanisms underlying MS and potentially lead to more effective therapeutic strategies. These findings highlight the possibility of exosomes as Ms biomarkers and potential therapeutic targets.

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