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Crossing the Blood-Brain Barrier

Mechanisms, Evidence, and Limits of EV-Mediated CNS Transit

Paper 04 in the CFT Advantage Series

WHITE PAPER

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Abstract

The blood-brain barrier (BBB) is one of the most stringent physiological filters in human biology, excluding approximately 98 percent of small-molecule drugs and effectively all large-molecule biologics from the central nervous system (Pardridge, 2005; Terstappen et al., 2021). This selectivity protects the brain but simultaneously constrains the delivery of molecules that might support normal neurological function. A growing body of pre-clinical research indicates that certain extracellular vesicle (EV) populations have demonstrated capacity to traverse the BBB through receptor-mediated transcytosis, adsorptive transcytosis, and lipid-raft-mediated pathways. This paper reviews the structural basis of BBB selectivity, the mechanisms by which EVs may cross it, the surface markers that appear to mediate transit, and the pre-clinical evidence of CNS delivery in animal models. The EVs discussed here form one fraction of the wider cell-free secretome. Particular attention is paid to the autologous case, where EVs derived from the patient's own biological material may interact more favourably with the patient's own endothelium, although direct comparative evidence remains limited. Throughout, we emphasise that almost all current evidence is pre-clinical, that brain accumulation in animal studies is typically modest, and that clinical evidence for EV-based neurological applications in humans remains very limited. The paper closes with a discussion of what these findings mean, and do not yet mean, for physicians and patients considering autologous cell-free therapy in the context of brain health.

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1. Introduction: The Blood-Brain Barrier Challenge

The central nervous system (CNS) is uniquely protected by the blood-brain barrier, a highly selective anatomical and biochemical interface that restricts the passage of most circulating substances into brain tissue. This protection is essential for neurological homeostasis: it shields neurons from the wide range of metabolites, hormones, immune cells, and pathogens circulating in peripheral blood. The same selectivity, however, presents one of the most formidable obstacles in modern therapeutics. Approximately 98 percent of small-molecule drugs and effectively all large-molecule biologics fail to cross the BBB at therapeutically meaningful concentrations (Pardridge, 2005; Terstappen et al., 2021), and this remains a primary reason why the pipeline of CNS-active treatments has lagged behind progress in oncology, cardiology, and metabolic disease. Barrier integrity is not static: it declines with age and in the presence of neuroinflammation, so permeability in older individuals may differ from that in younger ones.

In recent years, extracellular vesicles (EVs) have attracted attention as candidate vehicles for biological cargo that the BBB would otherwise exclude. Pre-clinical evidence indicates that certain EV populations can traverse the barrier through receptor-mediated and related transcytosis pathways, opening a route for delivery of nucleic acids, proteins, and lipid signals into brain tissue. The mechanism is biologically distinct from passive diffusion or paracellular leakage: it appears to involve specific interactions between vesicle surface proteins and endothelial receptors, followed by intracellular transit and release on the brain side.

This paper focuses specifically on the EV fraction of cell-free preparations. CFT preparations may also contain soluble proteins, lipids, metabolites, and other paracrine signals whose BBB behaviour may differ from that of vesicle-associated cargo, and which sit outside the scope of the present review. Within that scope, we examine the structural and functional properties of the BBB, the mechanisms by which EVs may traverse it, the surface markers that appear to facilitate transit, and the pre-clinical evidence supporting CNS delivery. We also discuss the autologous case, where EVs derived from the patient's own cells may interact with the endothelium as self rather than as foreign particles. Throughout, we emphasise that the present evidence base is overwhelmingly pre-

clinical and that mechanistic insights from animal studies do not yet constitute clinical evidence in humans. Our intent is to clarify the current state of knowledge while making the remaining evidence gaps explicit.

What this paper does not claim

This paper does not claim that CFT treats, prevents, cures, or mitigates any neurological disease. It does not claim that EV BBB transit in animal models proves human CNS efficacy. It reviews pre-clinical evidence that certain EV populations can cross the BBB and explains why that mechanism may be relevant to future research into autologous cell-free approaches.

2. The BBB: Structure, Function, and Selective Impermeability

2a. Tight Junctions and Endothelial Architecture

The blood-brain barrier is a highly specialised vascular interface. The physical foundation is the layer of endothelial cells lining the cerebral microvasculature, joined by tight junction proteins (claudins, occludin, ZO-1) that severely restrict paracellular transport. These tight junctions create a paracellular permeability orders of magnitude lower than that of capillaries in peripheral tissues, which is why hydrophilic substances that diffuse freely elsewhere in the body are effectively excluded from the brain.

The endothelial layer does not stand alone. Pericytes embedded in the basement membrane and perivascular astrocytes provide biochemical and structural cues that maintain barrier integrity. The perivascular space is also populated by microglia and other immune cells. Together with the basement membrane and the surrounding neuropil, these elements form the neurovascular unit, an integrated structure that has evolved to defend the CNS against circulating pathogens, toxins, and large hydrophilic molecules while still permitting the selective entry of nutrients.

2b. Active Efflux and Selective Transport

Selectivity is reinforced biochemically by active efflux. P-glycoprotein, breast cancer resistance protein (BCRP), and other ABC-family transporters expressed on the luminal face of brain endothelial cells actively pump many lipophilic and xenobiotic compounds back into the bloodstream after they have entered the cell membrane. This efflux is a major reason why drugs that look BBB-permeable on simple physicochemical grounds (small, lipophilic, neutral) still fail to accumulate in brain tissue at useful concentrations.

Despite this layered impermeability, the BBB does permit the selective entry of essential molecules through several routes: carrier-mediated transport for glucose, amino acids, and select nucleotides; receptor-mediated transcytosis for transferrin, insulin, and certain growth factors; and adsorptive-mediated transcytosis for cationic peptides and other charged ligands. Adsorptive transcytosis, in which charged surfaces bind nonspecifically to luminal membrane domains and trigger internalisation, is particularly relevant to understanding how EVs may cross the barrier without engaging a single dedicated receptor.

3. Mechanisms of EV Transcytosis Across the BBB

3a. Receptor-Mediated Transcytosis

The most extensively studied pathway for EV transit is receptor-mediated transcytosis. In this process, ligands on the EV surface bind specific receptors on the luminal face of the BBB endothelium, triggering clathrin-mediated or caveolin-mediated endocytosis. The vesicle is then trafficked through the endothelial cell and released on the abluminal side into the perivascular space. Receptor-mediated transcytosis is selective and saturable, behaviour characteristic of receptor-dependent pathways, and represents the same general mechanism the BBB uses to admit endogenous proteins such as transferrin and insulin.

Pre-clinical work has identified several receptor systems through which EVs may engage the endothelium. Transferrin receptor, low-density lipoprotein receptor-related protein 1 (LRP-1), and members of the integrin family are among the candidates implicated by in vitro and in vivo studies (Saint-Pol et al., 2020; Banks et al., 2020). The relative contribution of each receptor is likely to vary with the source cell type and the surface composition of the vesicle population.

3b. Adsorptive Transcytosis

A second, complementary mechanism is adsorptive transcytosis, in which charged or hydrophobic domains on the EV surface engage the luminal membrane non-specifically and trigger endocytosis without a dedicated receptor. This mode may be relevant for unmodified EVs, including autologous preparations, although direct comparative data remain limited. The Matsumoto et al. (2017) study of erythrocyte-derived vesicles, for example, attributes BBB transit to adsorptive-mediated transcytosis rather than a single receptor pathway, illustrating that vesicles produced endogenously in the bloodstream can engage the endothelium through this route.

3c. Lipid-Raft and Integrin-Mediated Pathways

A third uptake route involves lipid rafts, cholesterol- and sphingolipid-enriched membrane microdomains that promote endocytosis independent of clathrin or caveolin. EV membranes are enriched in lipid-raft components, and pre-clinical data indicate that these structures can support cellular uptake in barrier endothelium and elsewhere. In parallel, integrin-mediated adhesion appears to contribute. EVs commonly express integrins such as alpha-v-beta-3, alpha-v-beta-5, and alpha-4-beta-1, which can engage endothelial counter-receptors and extracellular matrix components, slowing the vesicle at the vessel wall and increasing the probability of internalisation (Yáñez-Mó et al., 2015).

These pathways are not mutually exclusive. EVs may engage multiple routes simultaneously or sequentially, with the dominant mechanism set by EV size, source cell, surface protein composition, and the inflammatory state of the BBB, which can itself vary with age and disease. Banks et al. (2020) showed that exposure to lipopolysaccharide, a model of systemic inflammation, increased uptake of several EV populations into the brain independently of overt barrier disruption, suggesting that the transcytosis machinery is dynamically modulated rather than fixed.

4. The Role of EV Surface Markers in BBB Crossing

4a. Tetraspanins and Integrins

The surface proteome of an extracellular vesicle is the interface that determines whether, and how, it interacts with the BBB endothelium. Tetraspanins (CD9, CD63, CD81) are among the most highly enriched proteins on EV surfaces. They span the membrane four times, organise into networks with other surface proteins, and create platforms for receptor engagement. Pre-clinical work indicates that tetraspanins contribute to EV uptake by endothelial cells, although the precise mechanism (whether through direct ligand binding, lateral organisation of integrins, or both) is still being characterised.

Integrins represent a second important class of EV surface molecules. The integrin profile of an EV population varies with the source cell type, and pre-clinical evidence suggests that integrin patterns can bias biodistribution toward specific tissues. In the context of the BBB, this raises an empirically tractable question: do autologous EVs derived from the patient's own cells display integrin profiles that interact more favourably with the patient's own endothelium than allogeneic preparations? Direct comparative data are limited, but the question is mechanistically plausible and worth dedicated investigation.

4b. Phosphatidylserine and Membrane Recognition

Phosphatidylserine (PS), a negatively charged phospholipid, is externalised on the outer leaflet of EV membranes. PS is recognised by several endothelial receptors, including TIM-4 and members of the phosphatidylserine-receptor family, and serves as a context-dependent uptake signal. Some evidence suggests that PS-mediated interaction enhances BBB penetration, while in other contexts it may direct vesicles toward macrophage clearance in liver and spleen. The net biodistribution effect appears to depend on the surface protein context that accompanies PS exposure rather than on PS alone (Wiklander et al., 2015).

Autologous EVs carry an additional, less studied feature at this interface: their broader surface proteome reflects the patient's own MHC and self-antigen repertoire. Recipient endothelium and tissue cells encounter them in the context of self markers. In allogeneic preparations, the same tetraspanins and integrins are present, but the surrounding self-antigen context is foreign, and the vesicle is exposed to the same opsonisation and complement-mediated clearance that the immune system applies to other allogeneic biological material. The implication is that the surface markers reviewed above may behave differently in autologous and allogeneic contexts even when the markers themselves are nominally the same. The size and consistency of any such difference for BBB transit specifically remains to be quantified.

5. Pre-Clinical Evidence of EV-Mediated CNS Delivery

5a. Foundational Studies

Multiple pre-clinical studies have demonstrated that EVs administered intravenously can penetrate the BBB and distribute into brain tissue in animal models. The seminal study by Alvarez-Erviti et al. (2011) used dendritic-cell-derived exosomes (a subset of small EVs) engineered to display the RVG (rabies virus glycoprotein) targeting peptide. Systemic injection in mice produced neuron-specific siRNA delivery and significant knockdown of BACE1, a neural target used in a mouse proof-of-principle study. The work established proof of principle that small EVs can deliver biologically active nucleic acid cargo across the BBB and into specific neural cell populations in vivo. Beyond engineered constructs, EVs from mesenchymal stromal cells have been reported to improve functional recovery in animal models of traumatic brain injury and stroke (Zhang et al., 2015). CFT is administered intravenously; other routes, including intranasal delivery, have also been explored pre-clinically in the wider literature.

Subsequent work has extended these findings in two directions. Saint-Pol et al. (2020) reviewed the literature on EV-BBB transit and described how endogenous and exogenous EVs engage receptor-mediated and adsorptive pathways at the cerebral endothelium. Banks et al. (2020) examined the brain pharmacokinetics of ten distinct EV populations derived from different cell sources, spanning

mouse and human and cancerous and non-cancerous origins. Here populations refers to preparations from different source cells, not to size sub-classes such as small and large EVs. All ten populations crossed the BBB, but the rate varied more than tenfold between them, and was modulated by systemic inflammation and by inhibitors that selectively block adsorptive transcytosis. The study showed that BBB transit is a population-specific property of the EV, not a uniform property of EVs as a class.

Matsumoto et al. (2017) added a different kind of evidence. Working with erythrocyte-derived extracellular vesicles, they documented vesicle transit across the BBB by adsorptive-mediated transcytosis in the context of neurodegeneration-related protein transfer. Whatever the implications for disease pathology, the experiment confirms that vesicles produced by ordinary blood cells, without any engineering, can cross the cerebral endothelium under physiological conditions.

5b. Caveats and Limitations of the Pre-Clinical Record

Several caveats accompany this body of work. The majority of studies use engineered or artificially loaded EVs, often from non-autologous sources such as dendritic cells, hepatocytes, or mesenchymal stem cells. Few studies directly compare autologous vesicles to other sources in the same animal model. Brain accumulation, while reproducible, is typically modest, on the order of 1 to 3 percent of the injected dose in the published biodistribution literature (Wiklander et al., 2015; Banks et al., 2020), and the duration of vesicle residence in brain tissue is poorly characterised.

Rodent BBB models also do not fully reproduce human BBB physiology. Differences span transporter expression, endothelial biology, neurovascular-unit architecture, and immune surveillance, and the disease biology that motivates many of these studies is itself only partially recapitulated in rodent systems. Larger-animal models, such as sheep and piglet, approximate human BBB physiology more closely and are increasingly used to bridge this gap. For these reasons, BBB transit observed in rodents should be treated as evidence of biological plausibility rather than as proof of human CNS delivery. Clinical data demonstrating EV-mediated CNS delivery and biological activity in human subjects remain very limited.

6. The Autologous Case in CNS Delivery

A defining feature of cell-free therapy (CFT) is that the extracellular vesicles, which form one fraction of the wider cell-free secretome administered to the patient, are derived from the patient's own cells. In the context of BBB crossing, this autologous origin produces several mechanistically plausible features. First, autologous EVs display a self surface proteome (including the patient's own MHC class I) that is encountered by the patient's endothelium in a self context. This raises the hypothesis that autologous EVs could interact more favourably with patient endothelium than allogeneic preparations, but direct evidence for superior BBB internalisation is currently limited. The autologous origin may also reduce some forms of immune recognition compared with

allogeneic or xenogeneic material, although systemic clearance by liver, spleen, and macrophage networks remains a major issue for EV preparations of all origins. Second, autologous EVs would not be expected to carry exogenous pathogen-associated molecular patterns (PAMPs, molecular signatures the immune system recognises as non-self) if produced under appropriate controlled conditions, and would therefore be less likely than contaminated or improperly handled material to trigger innate immune activation in the perivascular space. Third, autologous preparations may reduce donor-mismatch immunogenicity associated with allogeneic and xenogeneic material, including the potential risk of neutralising antibody formation across repeated administrations.

These features are mechanistically plausible, but the empirical record is thin. Direct head-to-head comparisons of autologous and non-autologous EV BBB crossing in well-controlled animal models are rare, and most translational EV neuroscience has focused on engineered delivery vehicles rather than on patient-derived material. The autologous case represents an important and largely open research area within EV neurobiology.

Any immunological advantages of autologous EVs may also extend beyond the moment of barrier transit. If autologous vesicles successfully reach CNS tissue, their recognition in a self context may reduce the likelihood of local glial activation and support integration into the perivascular niche. This could in principle enhance the durability and tissue residence of cargo delivery. Like the upstream features, this hypothesis remains to be tested in well-designed comparative studies.

7. Implications for Physicians and Patients

Physicians evaluating cell-free therapy in the context of brain health should treat the BBB-crossing literature for what it currently is: a growing pre-clinical body of work that establishes biological plausibility for EV transit, but does not establish clinical efficacy in any neurological condition. In conversations with patients, the responsible framing is that certain EV populations have been shown in animal studies to engage the cerebral endothelium through several recognised transcytosis pathways and to accumulate in brain tissue, and that research is ongoing to characterise the biological consequences of that accumulation in humans.

Three points are worth keeping in mind during such conversations. First, brain accumulation in animal studies is modest in absolute terms (on the order of 1 to 3 percent of injected dose), and the field has not yet established the threshold of accumulation required for biologically meaningful effects in humans. Second, the BBB-crossing literature concerns biological delivery of vesicles into brain tissue; it does not, by itself, demonstrate that any specific neurological outcome can be achieved. Third, autologous EVs occupy a different regulatory and safety position than engineered or allogeneic vehicles, and their theoretical immunological risk profile may be more favourable, but in major regulated markets such as the United States, neither autologous EVs nor any other EV preparation is currently approved for the treatment, cure, or prevention of neurological disease.

A scientifically cautious position, supported by the current literature, is that autologous cell-free therapy is delivered with the goal of supporting normal biological signalling. Whether EV access to

BBB-protected tissues translates into measurable CNS effects in humans remains under investigation. The mechanistic basis for vesicle access to the CNS is documented in pre-clinical models. The translation of that mechanistic basis into specific neurological outcomes is the active research question that the field is addressing now.

8. Current Limitations and Future Directions

Despite the encouraging pre-clinical record, several limitations constrain the present understanding of EV-mediated CNS delivery. The field's current standardisation expectations are reflected in MISEV2023 (Welsh et al., 2024), which updates earlier guidance on EV nomenclature, separation, characterisation, and functional analysis, but methodological consistency across laboratories is still a work in progress. Much of the underlying biology has been characterised in rodent models whose BBB physiology differs from human in measurable ways. The cells within brain tissue that internalise EVs, and the cell-type-specific consequences of that uptake, are not yet fully mapped. The relative contribution of intact, fully functional BBB transcytosis versus transient regional permeability changes is also incompletely resolved.

Priority areas for future research include: continued adoption of MISEV2023-aligned isolation and characterisation protocols to support reproducibility; direct comparative studies of autologous and allogeneic EV transit in larger animal models with more human-like BBB properties; mechanistic dissection of the relative contributions of receptor-mediated, adsorptive, and lipid-raft pathways in vivo; improved imaging of EV biodistribution and residence time in CNS tissue; characterisation of the immunological consequences of EV interactions with the neurovascular unit; and ultimately, well-designed human studies powered to assess safety and biological effect in defined neurological contexts.

9. Conclusion

The blood-brain barrier remains one of the most significant physiological challenges in therapeutic delivery. A growing body of pre-clinical evidence indicates that certain EV populations possess capacity to traverse the BBB through receptor-mediated transcytosis, adsorptive transcytosis, and lipid-raft-mediated pathways. This capacity appears to depend on specific surface features (tetraspanins, integrins, and phosphatidylserine) that engage endothelial counter-receptors and trigger internalisation and transit.

Multiple pre-clinical studies document brain accumulation following systemic administration of EVs, including biologically active cargo delivery in animal models. These findings are encouraging, but several caveats apply: most studies have used engineered vesicles from non-autologous sources, brain accumulation is typically modest, and clinical evidence in humans remains very limited. The field also faces methodological challenges, including ongoing standardisation under MISEV2023 and the limits of rodent models as predictors of human BBB behaviour.

Autologous EVs may carry mechanistically distinct features in this context, including interaction with the patient's own endothelium in a self context, theoretically lower immunological risk, and the absence of exogenous pathogen-associated molecular patterns when produced under appropriate controlled conditions. The mechanistic case is plausible, but direct comparative data establishing superior BBB transit or brain bioactivity for autologous preparations relative to other sources are still sparse. The autologous case is one of the field's important open questions.

In summary, the emerging evidence for EV-mediated BBB crossing represents a meaningful advance against a long-standing therapeutic bottleneck. Continued pre-clinical and clinical investigation is warranted to determine whether autologous EV-based approaches can safely and meaningfully influence CNS biology in humans. Until that evidence base matures, the application of EV-based approaches to neurological contexts must be framed as an active research frontier rather than established clinical practice. Cell-Free Therapy supports normal biological function. The mechanisms by which autologous EVs may contribute to neurological health remain under investigation.

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