Molecular Therapy
Commentary

Extracellular vesicles: A promising therapy against SARS-CoV-2 infection

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https://doi.org/10.1016/j.ymthe.2023.03.033

Severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2) has infected over 650 million people and claimed the lives of nearly 7 million since the start of the pandemic. While SARS-CoV-2 is becoming endemic with several preventative therapies, an effective treatment against severe disease remains unavailable. Immunocompromised patients remain vulnerable given the limited efficacy of vaccinations and are at risk of respiratory failure, organ failure, and septic shock if infected.[1] The development of therapeutics to combat the progression and severity of SARS-CoV-2 infection presents an opportunity to explore innovative approaches to treating viral diseases. Novel therapeutic strategies aim to target the host response to hyper-inflammation and prevent the cytokine storm that is often associated with severe COVID-19 cases.[2]

In recent years, researchers have focused on intracellular secreted factors, such as extracellular vesicles (EVs), to improve and build upon the knowledge gained from cell-based research and spearhead the use as potential therapeutic agents for various diseases, including SARS-CoV-2. EVs are small membranous structures secreted by the cell membrane or the cell's internal recycling pathways and have emerged as a promising therapeutic strategy due to their involvement in a range of biological processes, including cell signaling, immune response, and disease progression. The objective of this analysis is to examine the potential efficacy of EV-based therapies in the treatment of SARS-CoV-2 severity, with a particular emphasis on their common mechanisms and suitability for future therapeutic use in human patients.

Severe SARS-CoV-2 acts via direct and indirect pathways to cause local and systemic injury. In the direct pathway, severe SARS-CoV-2 utilizes its spike protein to bind to angiotensin-converting enzyme 2 (ACE-2), allowing entry into cells. Cells in the nasopharyngeal tract and lungs are most prone to damage by SARS-CoV-2 due to higher cell surface expression of the ACE-2 receptor.[3,4] After direct cellular entry, SARS-CoV-2 replicates using host machinery, and viral-mediated damage results in the secretion of pro-inflammatory cytokine interleukin-6 (IL-6).[5] Clinically, this presents with anosmia and ageusia with rapid progression to dyspnea and respiratory failure, ultimately resulting in multi-organ damage.[6]

The virus also acts via an indirect pathway to induce systemic injury. SARS-CoV-2-infected cells can undergo pyroptosis, which leads to the release of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs).[7] This mobilizes antigen-presenting cells (APCs)—including dendritic cells and pulmonary macrophages—that recognize PAMPs and DAMPs and release pro-inflammatory cytokines and chemokines, including interferon (IFN)-γ, IL-6, IP-10, and IL-1β. IL-1β further drives the activation of pro-inflammatory pathways, resulting in the recruitment of neutrophils and cytotoxic T cells and the upregulation of cytokines—mainly IL-6. Hypoxia induced by SARS-CoV-2 triggers further IL-6 secretion.[8] IL-6 also modulates its own expression by upregulating the production of IL-10 (anti-inflammatory). However, in the presence of SARS-CoV-2, there is significantly greater IL-6 production, resulting in a net pro-inflammatory state. Dysregulation of the innate immune response leads to increased inflammation and end-organ damage.[9,10]

One promising treatment modality for severe SARS-CoV-2 infection is EVs. EVs can secrete proteins and anti-inflammatory molecules that can modulate the host immune response. Additionally, EVs may act as a negative regulatory element in the transmission of viral infection.[11] Given these properties, EVs act at multiple points within the direct and indirect pathways to inhibit the inflammatory cascade (Figure 1). EVs can inhibit viral replication and thereby decrease direct viral injury.[12] Their ability to block IL-6, IL-6 precursors (IL-1β), and inflammatory cytokines (tumor necrosis factor α [TNF-α], IL-8, and MIP-2) at multiple points within the pathway[13,14] while upregulating IL-10 results in downregulation of cytokine production and reduction in systemic injury.

Given their ability to act on multiple pathways, EVs can provide a more comprehensive and effective approach to treating complex diseases.[15] To highlight the therapeutic properties of EVs, we conducted a review of 9 studies reporting the effects of EV therapy on lung injury models (Table 1). The reported outcomes of the reviewed EV therapies in experimental models (Table 1) indicate lung injury recovery, improved respiratory function, and overall survival. This was achieved by (1) reducing pro-inflammatory cytokines, (2) enhancing anti-inflammatory cytokines, (3) decreasing neutrophil infiltration, and (4) increasing macrophage polarization to the anti-inflammatory M2 phenotype. EV administration also resulted in downregulation of IL-1β, TNF-α, IL-6,[16–18] MIP-1,[18,19] MIP-2, and CXCL2,[18,20] in addition to upregulation of IL-10.[16,21,22] Moreover, EVs

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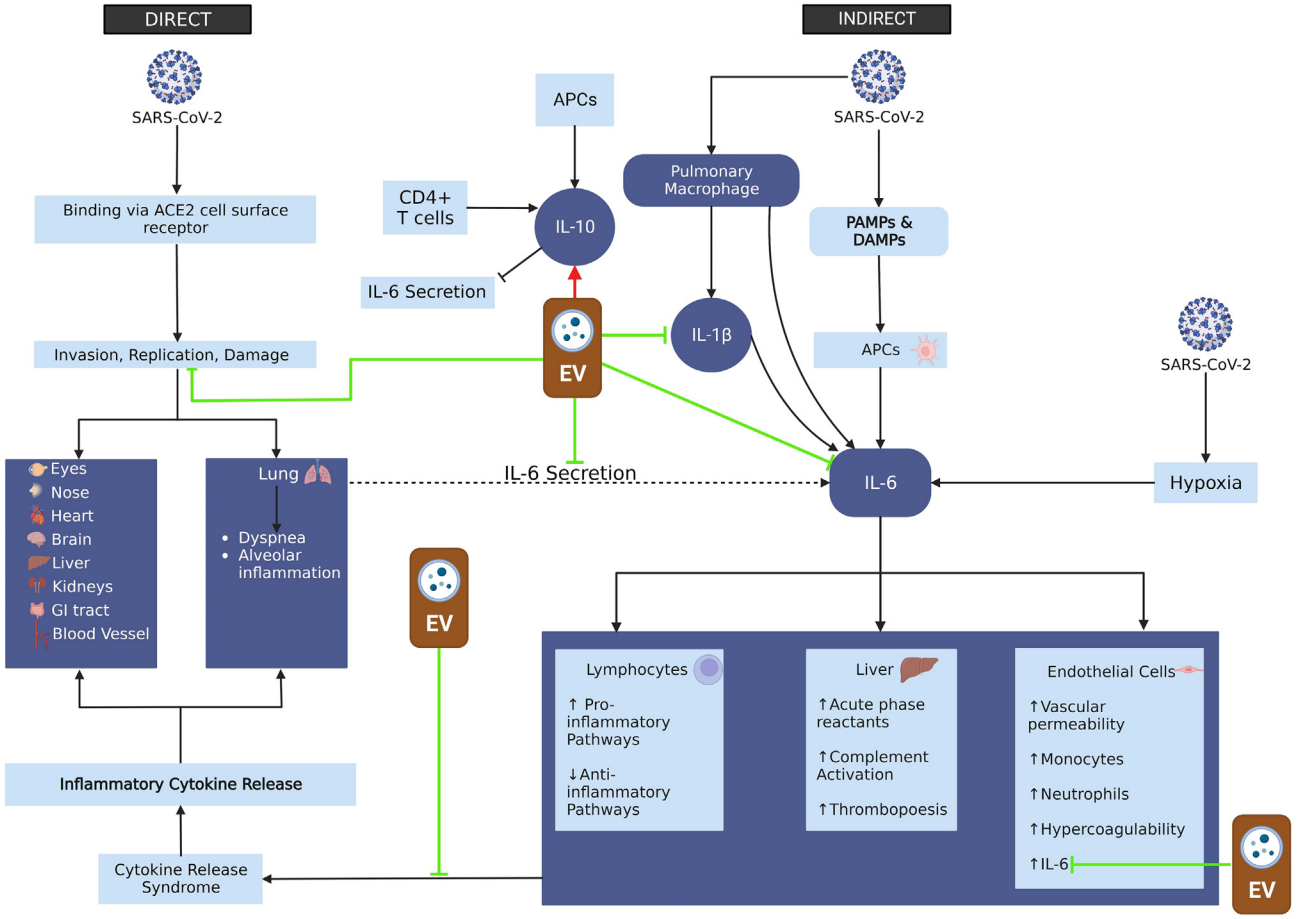

Figure 1. Mechanistic efficacy of extracellular vesicles against severe SARS-CoV-2 infection

SARS-CoV-2 acts through the direct and indirect pathway to induce systemic inflammatory injury. Extracellular vesicles secrete proteins and anti-inflammatory molecules to effectively block inflammatory mediators and pathways at multiple points along the pathways, resulting in downregulation of inflammation, cytokine release, and systemic damage.

reportedly preserved alveolar structure,[19] reduced alveolar wall thickness,[18,21,23] and inhibited virus-induced apoptosis in lung epithelial cells.[22] Finally, the ability of mesenchymal stem cell (MSC)-EVs to transfer cargo, such as miR-27a-3p, increased M2 macrophage polarization, effectively reducing TNF-α[17] and inhibiting lung fibrosis.[24]

The translation of EV-based therapies from acute lung injury models to human clinical studies have significant potential for the treatment of SARS-CoV-2-induced acute respiratory distress syndrome (ARDs). Preclinical models of lung injury have shown upregulated inflammatory responses and migration of neutrophils and macrophages to pulmonary tissues (Table 1). This was verified pathologically and shown to be due to an upregulation of IL-6. In preclinical models, EVs have demonstrated an ability to dampen inflammation and reduce T cell proliferation caused by SARS-CoV-2, establishing the rationale for present clinical trials.[25] Several EV-based therapies have entered clinical trials with the aim of assessing safety, efficacy, administration route, and optimal dosing in various respiratory conditions. A complete list of ongoing clinical trials on the use of EV-based therapeutics in COVID-19 treatment is shown in Table 2. Due to the rapid spread of COVID-19 and lack of effective therapies, several studies were approved on an emergency basis by ethical committees. In a study where amniotic fluid-derived EVs were administered to high-risk patients with mild-to-moderate COVID-19, results showed a significant decrease in CRP, IL-6, and TNF-α, as well as stabilized absolute lymphocyte count (ALC).[26] Additional clinical testing performed on three severely ill patients with COVID-19 revealed a decrease in inflammatory biomarkers and improvements in patient clinical status and respiratory function.[27] Both studies were completed without any adverse events or safety concerns.

The ongoing COVID-19 pandemic caused by SARS-CoV-2 highlights the critical need for effective therapies to mitigate disease progression and reduce severity. EV-based therapeutics have shown the capacity to attenuate the hyper-inflammatory response caused by SARS-CoV-2 and promote repair

Table 1. Articles reporting the effects of EV therapy on lung injury models

Source of EVs	Model	Sample	EV effects on cytokines and inflammatory molecules	Pathological outcomes	Source
Adipose MSCs	sepsis-induced ALI: *in vivo* mouse	lung tissueblood	↓IL-6, ↓TNF-α, ↓IL-1β, ↑EBI3-protein, ↑P28-protein↓IL-27	reduction in pulmonary inflammation and lung tissue injury (↓macrophage infiltration), increased survival rate	Wang et al.[16]
Amniotic fluid	BPD: *in vivo* rat	lung tissue	↓IL-1α, ↓IL-1β, ↓MCP-1, ↓MIP-1α	significant decrease of pulmonary hypertension, preservation of alveolar structure, reduction in vascular remodeling, suppression of lung inflammation, reduction of macrophage infiltration	Bellio et al.[19]
Adipose MSCs	ALI: *in vivo* mouseALI: *in vitro* mouse	lung tissueBALBMDMs	↓TNF-α, ↓IL-1β, ↓IL-6, ↑IL-10, ↓iNOS, ↓NF-κB↓TNF-α, ↓IL-1β, ↓iNOS, ↑ YM-1, ↑MRC-1, ↑mi-27-a-3p	reduction of pulmonary endothelial barrier, inflammation (↓ pro-inflammatory cytokines, ↑ anti-inflammatory cytokines, ↓ neutrophils), and alveolar septal thickening	Wang et al.[17]
Adipose MSCs	ALI: *in vivo* mouseALI: *in vitro* mouse	lung tissueBALBMDMs	↓IL-1β, ↑IL-10↓IL-6, ↑↓IL-1β, ↓TNF-α, ↓iNOS, ↑TGF-β1, ↑YM-1	reduction in inflammation (↓ neutrophils, ↓macrophage recruitment) and alveolar wall thickness	Huang et al.[21]
Bone marrow MSCs	ALI: *in vivo* mouseALI: *in vitro* mouse	BALRAW267.4	↓MIP-2, ↓TNF-α, ↑LTB4↓MRP1-protein, ↑miR-145	antimicrobial effect (↑ monocyte phagocytosis, ↓bacterial levels), reduction of inflammation (↓leukocytes, ↓neutrophils)	Hao et al.[20]
Umbilical cord jelly MSCs	influenza-induced ALI: *in vitro* human	AEC	no particular mechanism studied	restoration of alveolar fluid clearance, reduction alveolar protein permeability	[29]
Umbilical cord EPC (rich in miR-126)	ALI: *in vivo* mouseALI: *in vitro* human	lung tissueBALAEC	↓TNF-α, ↓IL-1β, ↓IL-6, ↓IFN-γ, ↓MIP-1↓MIP-2, ↓MIG, ↓IP-10, ↓MPO↑Claudin1, ↑Claudin4, ↑Occludin	reduction of inflammation (↓ pro-inflammatory cytokines, ↑ anti-inflammatory cytokines, ↓ neutrophils), alveolar wall thickness, and hyaline membrane formation	Zhou et al.[18]
Whole blood	fibrosis: *in vivo* mouse	lung tissue	↓hydroxyproline	reduction of immune cell recruitment, alveolar wall thickness, and collagen deposition	Sun et al.[23]
Bone marrow MSCs	influenza-induced ALI: *in vivo* piginfluenza-induced ALI: *in vitro* pigs	lung tissueLECs	↓TNF-α, ↓CXCL10, ↑IL-10↓apoptosis	inhibition of viral replication, reduction of inflammation, decrease in virus-induced lung lesions, inhibited virus-induced apoptosis in lung epithelial cells	Khatri et al.[22]

AECs, alveolar epithelial cells; ALI, acute lung injury; BAL, bronchioalveolar lavage; BMDMs, bone marrow-derived macrophages; BPD, bronchopulmonary dysplasia; CXCL, chemokine (C-X-C motif) ligand; EPC, endothelial progenitor cell; EVs, extracellular vesicles; IFN, interferon; IL, interleukin; LECs, lymphatic endothelial cells; LTB4, leukotriene B4; MCP, monocyte chemotactic protein; MIG, monokine induced by gamma interferon; MIP, macrophage induced protein; MIR, microRNA; MPO, myeloperoxidase; MRC-1, mannose receptor C-type 1; MRP1, multidrug resistance associated protein 1; MSC, mesenchymal stem/stromal cell; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NOS, nitric oxide synthase (iNOS, inducible; eNOS, endothelial); RAW267.4, monocyte/macrophage lineage; TGF, transforming growth factor; TNF, tumor necrosis factor; YM-1, Chitinase 3-like 3, a macrophage protein.

of damaged lung tissue in preclinical models, with similar results when translated into SARS-CoV-2 patients. In addition, EVs may harbor therapeutic applications to tackle the prolonged symptoms of infection (long COVID) that are associated with prolonged overactivation and exhaustion of immune cells.[28] The overview presented in this work highlights the innovative use of EVs as a promising approach to address severe SARS-CoV-2 infections. Recent progress in clinical trials has also laid the groundwork for the development of effective EV-based therapies for a broad range of viral infections.

Table 2. List of clinical trials on the use of EV-based therapeutics in COVID-19 management

Reference[a]	Official title	Status
NCT04493242	Extracellular Vesicle Infusion Treatment for COVID-19 Associated ARDS (EXIT-COVID19)	completed
NCT05787288	A Clinical Study on Safety and Effectiveness of Mesenchymal Stem Cell Exosomes for the Treatment of COVID-19	recruiting
NCT04657458	Expanded Access for Use of bmMSC-Derived Extracellular Vesicles in Patients With COVID-19 Associated ARDS	available
NCT05116761	ExoFlo™ Infusion for Post-Acute COVID-19 and Chronic Post-COVID-19 Syndrome	not yet recruiting
NCT05354141	Bone Marrow Mesenchymal Stem Cell Derived EVs for COVID-19 Moderate-to-Severe Acute Respiratory Distress Syndrome (ARDS): A Phase III Clinical Trial	recruiting
NCT05228899	Zofin to Treat COVID-19 Long Haulers	recruiting
NCT04902183	Safety and Efficacy of Exosomes Overexpressing CD24 in Two Doses for Patients With Moderate or Severe COVID-19	recruiting
NCT05216562	Efficacy and Safety of EXOSOME-MSC Therapy to Reduce Hyper-inflammation In Moderate COVID-19 Patients (EXOMSC-COV19)	recruiting
NCT04798716	The Use of Exosomes for the Treatment of Acute Respiratory Distress Syndrome or Novel Coronavirus Pneumonia Caused by COVID-19 (ARDOXSO)	not yet recruiting
NCT05387278	Safety and Effectiveness of Placental Derived Exosomes and Umbilical Cord Mesenchymal Stem Cells in Moderate to Severe Acute Respiratory Distress Syndrome (ARDS) Associated With the Novel Corona Virus Infection (COVID-19)	recruiting
NCT04491240	Evaluation of Safety and Efficiency of Method of Exosome Inhalation in SARS-CoV-2 Associated Pneumonia. (COVID-19EXO)	completed
NCT04384445	Zofin (Organicell Flow) for Patients With COVID-19	active, not recruiting
NCT04657406	Expanded Access to Zofin for Patients With COVID-19	available

[a]Obtained from clinicaltrials.gov using "extracellular vesicles" or "exosomes" as search strings, with results restricted to COVID-19.

ACKNOWLEDGMENTS

Y.L. would like to acknowledge the mentors who have played a profound role in their career development and to the patients who inspire them to strive for greatness daily. Y.L. also wants to acknowledge MSSN for its support of scholarly endeavors. Funding: Funding information is not applicable.

AUTHOR CONTRIBUTIONS

Y.L., idea conception; primary author for design, framework, and refinement of severe SARS-CoV-2 model, EVs as a therapeutic agent against severe SARS-CoV-2; review of literature; summarizing and organizing of data; main manuscript writing contribution; participation in post-peer review manuscript revisions. G.G., primary author for introduction, extracellular vesicle therapy, and future direction and conclusion. M.J., main manuscript writing; literature review; addition of substantial, clinically oriented written content. G.P.M., main manuscript writing; literature review; addition of substantial, clinically oriented written content. A.V.d.K., main manuscript writing; literature review; addition of substantial, clinically oriented written content. M.I.M., editing and revision of manuscript. M.A.B., editing and revision of manuscript. T.d.R., editing and revision of manuscript. All authors read, reviewed, and approved the final manuscript.

DECLARATION OF INTERESTS

G.G., M.I.M., T.d.R., and M.A.B. are employees of Organicell Regenerative Medicine. M.I.M. is the Chief Science Officer, serves on the Organicell Regenerative Medicine Board of Directors, and holds equity in the company.

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