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Hyper-inflammatory responses in COVID-19 and anti-inflammatory therapeutic approaches

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Abstract

The coronavirus disease 2019 (COVID-19) is an ongoing global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Patients with severe COVID-19 exhibit hyper-inflammatory responses characterized by excessive activation of myeloid cells, including monocytes, macrophages, and neutrophils, and a plethora of pro-inflammatory cytokines and chemokines. Accumulating evidence also indicates that hyper-inflammation is a driving factor for severe progression of the disease, which has prompted the development of anti-inflammatory therapies for the treatment of patients with COVID-19. Corticosteroids, IL-6R inhibitors, and JAK inhibitors have demonstrated promising results in treating patients with severe disease. In addition, diverse forms of exosomes that exert anti-inflammatory functions have been tested experimentally for the treatment of COVID-19. Here, we briefly describe the immunological mechanisms of the hyper-inflammatory responses in patients with severe COVID-19. We also summarize current anti-inflammatory therapies for the treatment of severe COVID-19 and novel exosome-based therapeutics that are in experimental stages.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) was first found in patients with unidentified pneumonia in Wuhan, China, in December 2019 and rapidly spread worldwide (1). The World Health Organization (WHO) announced the COVID-19 outbreak to be a pandemic on 11 March, 2020. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a positive-sense single-stranded RNA virus with high sequence homology to bat coronaviruses (CoVs). Other CoVs have exhibited severe infections in humans. These include SARS-CoV-1 and Middle East respiratory syndrome (MERS)-CoV, which emerged in 2003 and 2012, respectively (2). SARS-CoV-2 uses its spike protein to bind the angiotensin-converting enzyme 2 (ACE2) receptor expressed on the cell membrane for entry into host cells (3, 4). Although SARS-CoV-2 is not as lethal as MERS-CoV and SARS-CoV-1 (5), its substantial spread has resulted in severe casualties and caused overwhelming pressure for the medical system worldwide.

Nearly 20% of patients with COVID-19 experience severe disease (6, 7). Accumulating evidence suggests that hyper-inflammatory responses of the host to SARS-CoV-2 infection lead to severe forms of COVID-19 (8, 9). Immunopathological features, such as excess infiltration of patients' lungs by macrophages and neutrophils, as well as increased serum cytokine and chemokine levels, are characteristics of severe COVID-19 (6, 10, 11). Anti-inflammatory therapies, including corticosteroids, are currently considered the standard of care in treating patients with severe COVID-19 (12). The clinical efficacy of other anti-inflammatory therapies is being investigated in various clinical trials.

Here, we review the mechanisms of hyper-inflammatory responses in COVID-19 and describe recent advances in anti-inflammatory therapies for COVID-19. We also highlight the potential roles of exosomes as novel anti-inflammatory therapeutics for the treatment of

COVID-19.

THE DYSREGULATED IMMUNE RESPONSE IN PATIENTS WITH COVID-19

Severe COVID-19 pathology results from massive initial viral replication that arises because the SARS-CoV-2 can evade and inhibit the host innate immune recognition system and interferon (IFN) responses (13). Type I IFNs (IFN-αs and IFN-β) and type III IFNs (IFN-λs) are the major first-line defenses against viruses (14), but SARS-CoV-2 has developed various strategies to evade and suppress the production and functions of type I and III IFNs and IFN-stimulated genes (ISGs) (15-17). These mechanisms allow SARS-CoV-2 to replicate robustly, leading to excessive activation of monocytes, macrophages, and neutrophils. Excessively activated myeloid cells then produce excessive pro-inflammatory cytokines and chemokines, resulting in hyper-inflammatory responses (13, 18-20).

Excessive neutrophil activation and infiltration

Patients with severe COVID-19 exhibit increased neutrophil counts with a high neutrophil-to-lymphocyte ratio as an independent risk factor for severity (21-23). A transcriptome analysis of bronchoalveolar lavage fluid (BALF) from patients with COVID-19 showed that SARS-CoV-2 infection induces excessive neutrophil infiltration compared to other forms of pneumonia (24). In addition, patients with severe COVID-19 exhibit increased tissue infiltration of neutrophils in the upper airways of the lungs (25) and the bronchoalveolar space (26, 27). A recent study found that NSP10 of SARS-CoV-2 interacts with the NF-κB repressor NKRF to induce IL-8 production, which augments IL-8-mediated chemotaxis of neutrophils and the over-exuberant host inflammatory responses in COVID-19 (28). Furthermore, increased formation of neutrophil extracellular traps (NETs), which are net-like

structures composed of DNA, antimicrobials and oxidant enzymes released by neutrophils, exacerbates lung injury and inflammation in patients with severe COVID-19 (29, 30). These findings imply that the dysregulated activation of neutrophils contributes to hyper-inflammatory responses in severe cases of COVID-19.

Dysregulated activation of macrophages and monocytes

Macrophage activation, especially in the lungs, plays a key role in the progression of dysregulated immune responses in patients with severe COVID-19. Single-cell RNA sequencing (scRNA-seq) analysis of BALF from patients with COVID-19 has shown elevated numbers of pro-inflammatory macrophages in the lungs of such patients (24, 26). The lung macrophages of such patients have shown increased expression of pro-inflammatory cytokine genes, such as *IL1B*, *IL6*, and *TNF*, as well as chemokine genes, such as *MCP1/CCL2*, *MIP1A/CCL3*, *MIP1B/CCL4*, and *MCP3/CCL7* (24, 26). Dysregulated activation of monocytes also contributes to severe progression of COVID-19. Elevated numbers of inflammatory monocytes have been identified in the blood of patients with severe COVID-19 (31, 32). A recent large-scale single-cell transcriptome atlas study claimed that monocytes in the peripheral blood are key contributors to the cytokine storm in such patients (32). This is supported by a study showing that inflammatory monocytes in the blood of patients with COVID-19 exhibit elevated gene expression related to classical M1 macrophages (33). Strikingly, scRNA-seq analyses showed that pro-inflammatory cytokines trigger the activation and expansion of circulating monocytes, suggesting positive feedback between the activation of monocytes and production of pro-inflammatory cytokines (33, 34). These observations collectively suggest that SARS-CoV-2 infection triggers dysregulated activation of macrophages and monocytes, resulting in the secretion of a plethora of pro-

inflammatory cytokines and chemokines.

Mechanistic model of hyper-inflammation in severe COVID-19

A mechanistic model that explains the contribution of delayed IFN responses to the exacerbated inflammatory response in patients with severe COVID-19 has been proposed (Fig. 1) (13, 20). After SARS-CoV-2 infection of respiratory epithelial cells, the virus efficiently evades host innate immune recognition and IFN responses by blocking the type I and III IFN responses. The viral load rapidly increases, and myeloid cells, such as monocytes and macrophages, are stimulated by viral components via Toll-like receptors (TLRs). Then the monocytes and macrophages produce type I and III IFNs. Positive feedback occurs between the production of IFNs and chemokines and the accumulation and activation of monocytes and macrophages, thus producing large amounts of pro-inflammatory cytokines, such as TNF, IL-6, and IL-1β. This model explains how delayed but exaggerated IFN responses contribute to hyper-inflammation and severe progression of COVID-19.

CURRENT ANTI-INFLAMMATORY THERAPIES FOR COVID-19

Accumulating evidence of hyper-inflammation in patients with severe COVID-19 has provoked the development of anti-inflammatory therapies. Currently, more than 6000 clinical trials testing various therapeutics for treating COVID-19 are registered at clinicaltrials.gov, many of them with anti-inflammatory therapeutics. Various anti-inflammatory agents, such as corticosteroids, IL-6R inhibitors, and JAK inhibitors, have already been shown to be effective in ameliorating hyper-inflammation in COVID-19 (Table 1).

Corticosteroids

Glucocorticoids strongly inhibit the immune system. Glucocorticoids function as glucocorticoid receptor (GR) agonists. Binding of the glucocorticoids to the GR activates the receptor to exert anti-inflammatory effects, such as suppressing the production of pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, etc.) (35, 36). A controlled, open-label, randomized RECOVERY trial evaluated the efficacy of the glucocorticoid dexamethasone in hospitalized patients with COVID-19 (12). There were 2104 COVID-19 patients who received 6 mg of dexamethasone once daily for up to 10 days; these patients were compared with 4321 patients who received usual care (12). The trial demonstrated a significantly lower 28-day death rate in the dexamethasone group versus the usual care group (22.9% versus 25.7%; $p < 0.001$) (12). In particular, the use of dexamethasone lowered the 28-day death rate for those who received either invasive mechanical ventilation or oxygen alone at randomization, but not for those receiving no respiratory support (12), which implies that dexamethasone is effective only in severe patients with ongoing hyper-inflammation. Early meta-analyses also confirmed that therapeutic use of corticosteroids for COVID-19 is recommended in severe patients who require respiratory support or mechanical ventilation (37, 38).

IL-6R inhibitors

IL-6 is an important cytokine involved in the hyper-inflammatory response in patients with severe COVID-19, which prompted the use of selective IL-6 inhibitors for treatment of these patients. Tocilizumab, a recombinant humanized monoclonal antibody for IL-6 receptor (IL-6R), exerts therapeutic effects by blocking the binding of IL-6 to IL-6R (39). This treatment was previously found be effective against the cytokine release syndrome resulting from chimeric antigen-receptor T-cell therapy (40). Several studies have shown the therapeutic

effect of tocilizumab in treating severe COVID-19 by rapidly decreasing inflammatory markers, improving oxygenation, and reducing the death rate in COVID-19 patients who are on mechanical ventilation (41-43). Nevertheless, there is a debate regarding the therapeutic effect of tocilizumab in treating COVID-19. On the one hand, Gupta *et al.* conducted a multicenter cohort study of 4485 COVID-19 patients with intensive care unit (ICU) admission and reported that the risk of in-hospital death was lower in patients treated with tocilizumab in the first two days of ICU admission (44). On the other hand, Stone *et al.* did a randomized, double-blind, placebo-controlled trial with 243 COVID-19 patients with hyper-inflammation and concluded that tocilizumab was not effective at preventing intubation or death in moderately ill patients hospitalized with COVID-19 (45). However, Leaf *et al.* argued that the result was severely underpowered, by pointing out that the percentage of patients with primary outcomes such as intubation or death was 12.5% in the placebo group, which is far lower than the expected 30% (46). They claimed that the percentage of primary outcomes and number of patients enrolled (n = 243) would have made it nearly impossible for the trial to have demonstrated a therapeutic effect (46). Salama *et al.* also reported that tocilizumab did not improve survival in a randomized trial of hospitalized COVID-19 patients with pneumonia, but treatment reduced the likelihood of progression to mechanical ventilation or death (47). Similarly, in a randomized trial of 452 hospitalized patients with severe COVID-19 accompanied by pneumonia, Rosas *et al.* reported that the use of tocilizumab did not lead to improved clinical status or lower death rate than did a placebo at 28 days (48). Another monoclonal antibody for IL-6R, sarilumab, has also had controversial results in COVID-19 patients (49, 50). Thus, the therapeutic efficacy of the IL-6R inhibitor for COVID-19 patients should be carefully examined in further clinical studies.

JAK inhibitors

Inhibitors of the Janus kinases (JAKs) are powerful anti-inflammatory agents that effectively ameliorate various inflammatory diseases, such as rheumatoid arthritis (51). JAK inhibitors suppress the kinase activity of JAKs by competitively binding to the ATP-binding site of JAKs, thereby inhibiting signal transduction of a wide variety of cytokines (51). In a preclinical study, baricitinib, a clinically approved JAK1/JAK2 inhibitor, suppressed the production of pro-inflammatory cytokines and chemokines from lung macrophages and the recruitment of neutrophils in SARS-CoV-2-infected rhesus macaques (52). In addition, a double-blind, randomized, placebo-controlled trial evaluating the effect of baricitinib plus remdesivir in 1033 hospitalized adults with COVID-19 reported that this combination was more effective than remdesivir alone in reducing the recovery time and improving clinical status among patients with severe COVID-19 who were receiving high-flow oxygen or non-invasive ventilation (53). In a retrospective, uncontrolled patient cohort with moderate-to-severe COVID-19, treatment with baricitinib plus hydroxychloroquine demonstrated clinical improvement in 11 of 15 patients (54). In July 2021, baricitinib was approved by the US Food and Drug Administration as a single treatment for hospitalized patients with COVID-19.

Another JAK inhibitor, ruxolitinib, demonstrated clinical improvement in 18 critically ill COVID-19 patients with acute respiratory distress syndrome (ARDS) (55). In a prospective, multicenter, single-blind, randomized controlled phase II trial involving 43 patients, no significant difference was observed in ruxolitinib-treated patients compared to controls, though ruxolitinib recipients had faster clinical improvement (56).

EXOSOMES AS POTENTIAL THERAPEUTICS IN COVID-19

Characteristics of exosomes

Extracellular vesicles (EVs) are natural nanoparticles secreted by the cell. They are classified into three subgroups: exosomes, microvesicles, and apoptotic bodies, which have different biological properties in their biogenesis, content, and size (exosomes, 30~150 nm; microvesicles, 0.1~1 μm; and apoptotic bodies, 1~5 μm) (57, 58). Exosomes are enclosed by a single lipid bilayer which are generated by inward budding of vesicles into endosomes that mature into multivesicular bodies or by direct budding of lipid vesicles from the plasma membrane (59). Exosomes are known to be secreted by all cell types and are present in various body fluids (60-65). Exosomes participate in intercellular delivery of diverse biological molecules, such as nucleic acids (DNA, RNA), proteins, lipids, and carbohydrates. Many efforts have been made to apply exosomes for various therapeutic application via the engineering of exosomes or exosome-producing cells for incorporating active pharmaceutical ingredients (APIs) into exosomes and inducing targetability to specific cells or organs (66-68).

Current exosome-based therapeutics for treating COVID-19

Mesenchymal stem cells (MSCs), which are multipotent adult stem cells, are an easily accessible type of stem cell that are present in various human tissues. Considerable interest in MSCs has been raised for their therapeutic efficacy in tissue repair and in suppressing inflammation. Interestingly, enough research has shown that MSCs exert therapeutic effects by secreting EVs, not by a differentiation mechanism (69, 70). In line with this finding, MSC-derived exosomes have demonstrated regenerative potential, immune-modulatory functions, and anti-inflammatory effects (71).

Recent studies have highlighted the therapeutic potential of MSC-derived exosomes for

229 treating COVID-19. In a prospective, non-randomized, open-label cohort study, the efficacy
230 of exosomes derived from allogenic bone-marrow (BM) MSCs was evaluated in 24 COVID-
231 19 patients with moderate-to-severe ARDS (72). BM MSC-derived exosomes demonstrated a
232 survival rate of 83%, with 17 of 24 (71%) patients recovered showing no adverse effects
233 observed within 72 hours of exosome administration (72). In addition, improved respiratory
234 function (PaO_2/FiO_2) and reduced neutrophil count and acute phase reactants (i.e., C-reactive
235 protein, ferritin, and D-dimer) were observed (72). However, the clinical outcome of this
236 study must be carefully interpreted because little information was provided about the
237 characteristics, biological properties, or proposed biological or therapeutic actions of the BM-
238 MSC-derived exosomes used in this study (73). Four clinical trials, mostly phase 1 or 2,
239 evaluating the therapeutic effect of MSC-derived exosomes in COVID-19-associated
240 pneumonia are currently in progress (NCT04276987, NCT04798716, NCT04602442,
241 NCT04491240, Table 2).
242 Engineered exosomes demonstrating potent anti-inflammatory effects have potential to act
243 as effective immunomodulators to ameliorate the excessive inflammation observed in patients
244 with severe COVID-19. Recently, a clinical trial (NCT0474574, Table 2) conducted in Israel
245 completed a phase 1 trial, in which 30 patients with moderate or worse COVID-19 were
246 treated with CD24-expressing exosomes (EXO-CD24). More results are awaited regarding
247 the therapeutic effect of anti-inflammatory exosomes in relieving immunopathogenesis in
248 severe COVID-19.
249
250 **Therapeutic exosome platform technologies for efficient intracellular cargo delivery**
251 Various exosome engineering platform technologies that can generate exosomes armed with
252 therapeutic cargo have been developed. APIs could be “post-incorporated” into isolated

exosomes through exogenous methods, or “pre-incorporated” into exosomes through endogenous methods by modifying the exosome-producing cells (Fig. 2). Exogenous cargo loading methods involves loading APIs into exosomes through methods such as sonication, electroporation, freeze-thaw cycles, and extrusion (Fig. 2B) (74-79). However, a major caveat of these methods is damage to the exosomal membranes during the loading process (80). Endogenous cargo loading uses exosome-producing cells to load APIs into exosomes during natural exosome biogenesis. For example, biological agents can be endogenously incorporated into exosomes by genetically modifying the exosome-producing cells to overexpress the desired proteins or nucleic acids, which are then naturally loaded into exosomes. Macrophage-derived EVs loaded with IL-10 by transfecting the *IL10* gene to EV-producing cells demonstrated therapeutic efficacy in ischemia/reperfusion injury-induced acute kidney injury (AKI) by ameliorating the renal tubular injury and inflammation and driving M2 macrophage polarization via targeted delivery of EVs to macrophages (81). Endogenous cargo loading can be improved by inducing additional modification to the cargo, such as by anchoring the cargo onto the inner/outer membrane of exosomes via conjugation with membrane proteins of exosomes, such as PTGFRN (Fig. 2C) (82). However, the drawback of this approach is that APIs remain attached to the membrane of exosomes after delivery to the target cell, which may dramatically restrict its biological function. Alternatively, a novel technology called EXosomes for Protein Loading via Optically Reversible protein-protein interaction (EXPLOR) has been developed which could load non-anchored free-form proteins into exosomes using light-induced hetero-dimerizing modules, cryptochrome 2 (CRY2), and the N-terminal of CRY-interacting basic-helix-loop-helix 1 (CIBN) isolated from *Arabidopsis thaliana* (83). CRY2 and CIBN undergo hetero-dimerization in a blue light-specific manner, but reversibly and rapidly dissociate with each

other in the absence of blue light (84, 85). By fusing CRY2 with the cargo protein and CIBN with the exosomal membrane protein CD9, cargo proteins can be loaded into exosomes with high yield under blue light via the natural exosome biogenesis pathway (Fig. 2D).

EXPLOR technology has been applied to generate anti-inflammatory exosomes loaded with anti-inflammatory proteins that inhibit the NF-κB signaling pathway. Exosomes loaded with super-repressor IκB (srIκB), a dominant active form of IκBα, generated by EXPLOR technology has demonstrated a promising therapeutic effect in inflammatory diseases, such as sepsis (86-88). SrIκB is a degradation-resistant form of NF-κB-inhibiting protein IκBα, which blocks the nuclear translocation of NF-κB even when pro-inflammatory stimulus is present. Administration of exosomes loaded with srIκB (Exo-srIκB) to septic mouse models ameliorated the death rate and systemic inflammation by reducing the levels of circulating pro-inflammatory cytokines and alleviating acute organ injury (86). Intravital imaging revealed that the administered exosomes are taken up mainly by neutrophils and monocytes, which are attractive target cells for treating hyper-inflammation in COVID-19 (86). Exosomes are cleared by phagocytic cells, such as macrophages and neutrophils, after systemic injection, which makes these cells a primary target for exosomal therapeutics (89, 90). In an AKI model of C57BL/6 mice, Exo-srIκB treatment has demonstrated an anti-inflammatory effect by decreasing gene expression of pro-inflammatory cytokines and adhesion molecules in post-ischemic kidneys (88). In addition, Exo-srIκB administration affects the post-ischemic kidney immune-cell population, reducing the neutrophil, monocyte, and macrophage populations (88). As suppression of the NF-κB pathway has been implied as a potential therapeutic approach for treating patients with severe COVID-19 (91, 92), results demonstrating the therapeutic efficacy of Exo-srIκB in various inflammation-related disorders suggest a possibility of applying Exo-srIκB in the treatment of hyper-inflammation

in severe COVID-19.

SUMMARY AND PERSPECTIVE

Excessive activation of myeloid cells, such as monocytes, macrophages, and neutrophils, and exaggerated production of pro-inflammatory cytokines result in hyper-inflammation, leading to severe progression of COVID-19. Anti-inflammatory agents, such as dexamethasone and baricitinib, have shown promising results in ameliorating hyper-inflammation in patients with severe COVID-19, which implies that targeting hyper-inflammation is an appropriate strategy for ameliorating the severity of COVID-19. In addition, exosomes have arisen as a novel therapeutic modality for treating dysregulated inflammatory responses in COVID-19, either as a naïve form or a bioactive cargo delivery vehicle. Promising results of anti-inflammatory exosome therapeutics in ameliorating various inflammatory diseases have indicated the possibility of applying exosomes in the treatment of hyper-inflammation in COVID-19. Recent studies and clinical trials have reported a therapeutic potential of anti-inflammatory exosomes in treating patients with COVID-19. Nonetheless, more research is needed, as well as randomized clinical trials, with enough enrolled patients to verify the efficacy of exosomes in treating patients with severe COVID-19.

Therapeutics	Mechanisms of action and therapeutic efficacy
Corticosteroids	-Glucocorticoids exert anti-inflammatory effects by binding to glucocorticoid receptor. -Glucocorticoid dexamethasone reduced the 28-day death rate in patients with severe COVID-19 (12). -Dexamethasone is recommended for use in severe COVID-19 patients with ongoing hyper-inflammation.
IL-6R inhibitors	-IL-6R inhibitors are recombinant humanized antibodies for IL-6R that block the binding of IL-6 to IL-6R. -IL-6R inhibitors have controversial therapeutic efficacy in COVID-19.
JAK inhibitors	-JAK inhibitors suppress the kinase activity of JAKs by competitively binding to the ATP-binding site of JAKs. -Baricitinib was approved by the US FDA for the treatment of hospitalized patients with COVID-19.

Table 1. Mechanisms of action and therapeutic efficacy of current anti-inflammatory therapies for severe COVID-19

Therapeutic exosomes	Delivery method	Dosage	Phase	NCT number
Exosomes overexpressing CD24	Inhalation	10^{10} particles in 4 ml normal saline	2	NCT04969172
CovenD24 (exosomes overexpressing CD24)	Inhalation	10^{9}, 10^{10}particles	2	NCT04902183
Ardoxso (MSC-derived exosomes)	Intravenous infusion	2×10^{9}, 4×10^{9}, 8×10^{9} particles	1,2	NCT04798716
EXO 1, EXO 2 (MSC-derived exosomes)	Inhalation	0.5-2 $\times 10^{10}$ particles in 3 ml special solution	2	NCT04602442
EXO-CD24 (exosomes overexpressing CD24)	Inhalation	1×10^{8} - 1×10^{10} particles per 2 ml saline	1	NCT04747574
EXO 1, EXO 2 (MSC-derived exosomes)	Inhalation	0.5-2 $\times 10^{10}$ particles in 3 ml special solution	1	NCT04491240
CSTC-Exo (COVID-19-specific T cell-derived exosomes)	Inhalation	2×10^{8} particles in 3 ml	1	NCT04389385
MSC-derived exosomes	Inhalation	2×10^{8} particles in 3 ml	1	NCT04276987

Table 2. Ongoing clinical trials evaluating the efficacy of exosome therapeutics in COVID-19

Figure legends

Figure 1. Mechanistic model of hyper-inflammation in COVID-19

After respiratory epithelial cells are infected (A), SARS-CoV-2 proteins block viral-recognition signaling and type I and III interferon (IFN) responses (B). The viral load increases (C) and myeloid cells, such as monocytes and macrophages, are stimulated by viral components via Toll-like receptors, producing type I and III IFNs (D). IFNs further stimulate the production of chemokines and induce the accumulation and activation of monocytes and macrophages, thus producing excessive amounts of pro-inflammatory cytokines (E). This process can be amplified by a positive feedback mechanism.

Figure 2. Engineering methods for loading therapeutic agents into exosomes

The techniques for loading cargo into exosomes can be divided into four approaches. (A) Using naïve exosomes (e.g., MSC-derived exosomes) requires relatively simple techniques to generate therapeutic exosomes, but the drawback is the difficulty in controlling the bioactive molecules contained in the exosomes. (B) Exogenous cargo loading is based on the use of sonication, repeated freeze/thaw cycles, or electroporation to destabilize the integrity of exosomal membranes and thus allow drugs to be introduced into the exosomes. (C, D) Endogenous cargo loading spontaneously loads molecules of interest by hijacking the natural exosome biogenesis pathway. These techniques are divided into two approaches based on whether the cargo is anchored onto the exosomal membrane (C) or resides as a free form inside the lumen of the exosome (D).

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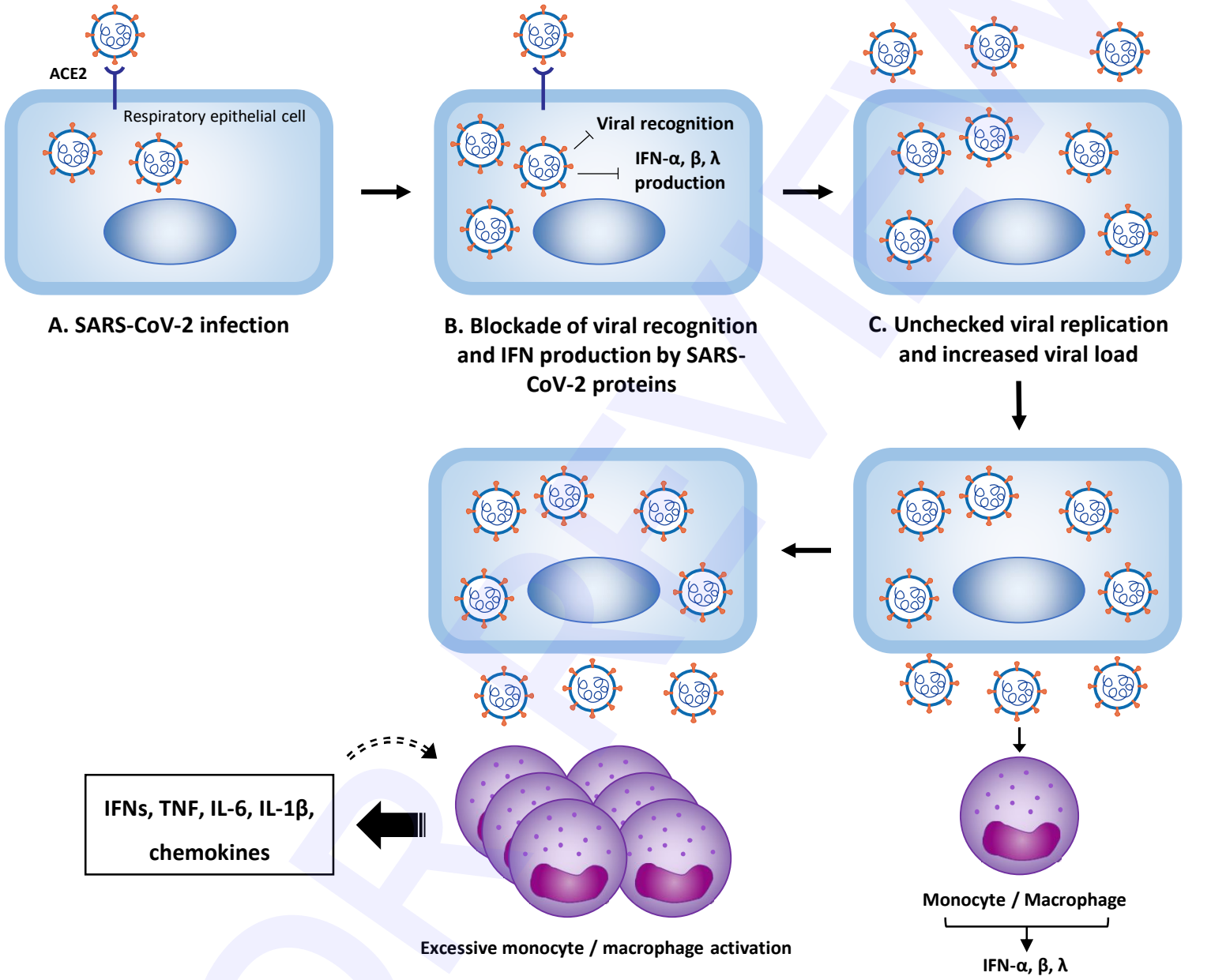
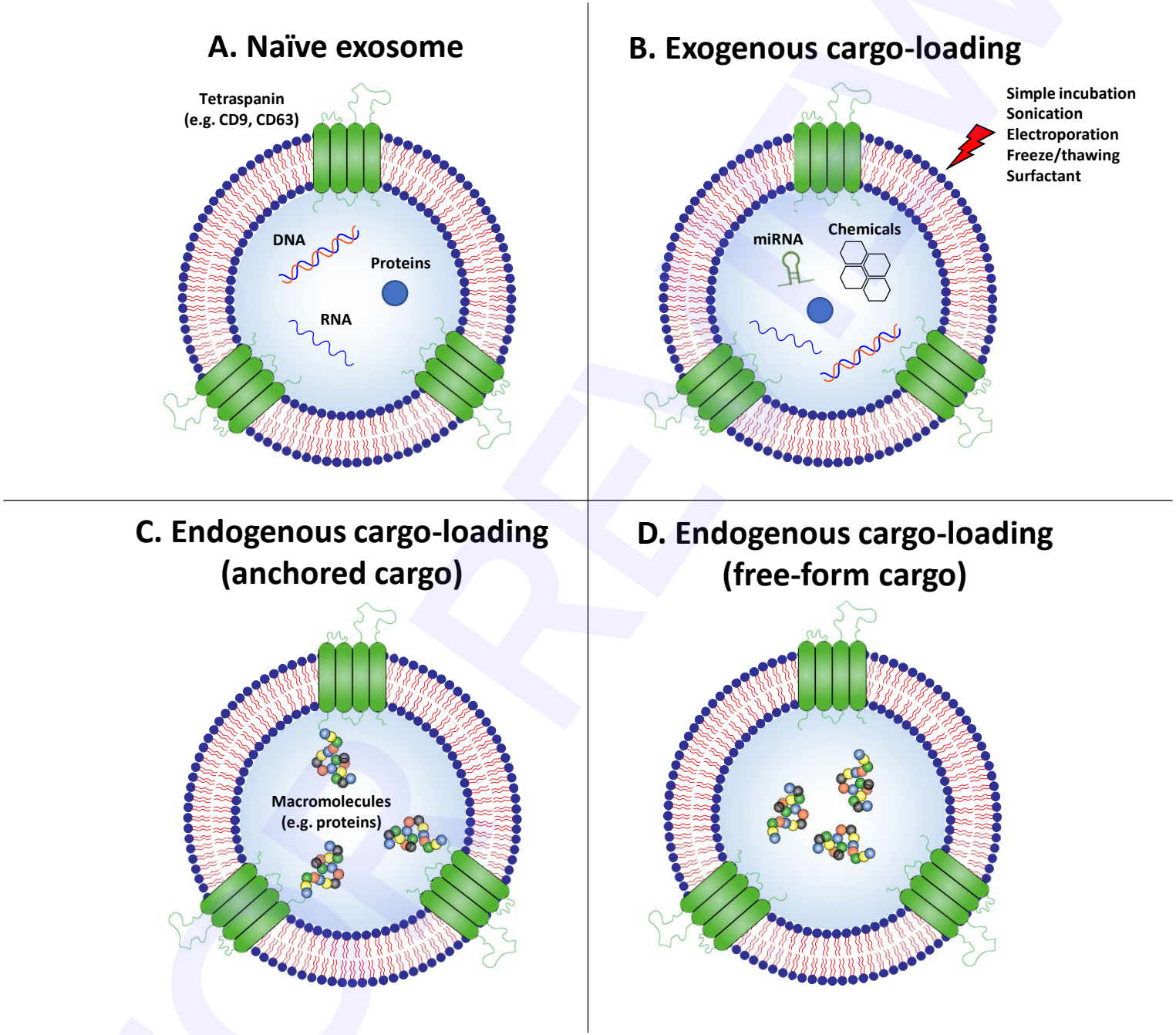

ACE2
Respiratory epithelial cell
A. SARS-CoV-2 infection
Viral recognition
IFN-α, β, λ production
B. Blockade of viral recognition and IFN production by SARS-CoV-2 proteins
C. Unchecked viral replication and increased viral load
Monocyte / Macrophage
IFN-α, β, λ
D. Delayed but exaggerated IFN production from innate immune cells
IFNs, TNF, IL-6, IL-1β, chemokines
Excessive monocyte / macrophage activation
E. Exacerbation of inflammation by IFNs, pro-inflammatory cytokines and chemokines


A. Naïve exosome
Tetraspanin
(e.g. CD9, CD63)
DNA
Proteins
RNA
B. Exogenous cargo-loading
Simple incubation
Sonication
Electroporation
Freeze/thawing
Surfactant
miRNA
Chemicals
C. Endogenous cargo-loading
(anchored cargo)
Macromolecules
(e.g. proteins)
D. Endogenous cargo-loading
(free-form cargo)

Therapeutics	Mechanisms of action and therapeutic efficacy
Corticosteroids	-Glucocorticoids exert anti-inflammatory effects by binding to glucocorticoid receptor. -Glucocorticoid dexamethasone reduced the 28-day death rate in patients with severe COVID-19 (12). -Dexamethasone is recommended for use in severe COVID-19 patients with ongoing hyper-inflammation.
IL-6R inhibitors	-IL-6R inhibitors are recombinant humanized antibodies for IL-6R that block the binding of IL-6 to IL-6R. -IL-6R inhibitors have controversial therapeutic efficacy in COVID-19.
JAK inhibitors	-JAK inhibitors suppress the kinase activity of JAKs by competitively binding to the ATP-binding site of JAKs. -Baricitinib was approved by the US FDA for the treatment of hospitalized patients with COVID-19.

Table 1. Mechanisms of action and therapeutic efficacy of current anti-inflammatory therapies for COVID-19

Therapeutic exosomes	Delivery method	Dosage	Phase	NCT number
Exosomes overexpressing CD24	Inhalation	10^{10} particles in 4 ml normal saline	2	NCT04969172
CovenD24 (exosomes overexpressing CD24)	Inhalation	10^{9}, 10^{10}particles	2	NCT04902183
Ardoxso (MSC-derived exosomes)	Intravenous infusion	2×10^{9}, 4×10^{9}, 8×10^{9} particles	1,2	NCT04798716
EXO 1, EXO 2 (MSC-derived exosomes)	Inhalation	0.5-2 $\times 10^{10}$ particles in 3 ml special solution	2	NCT04602442
EXO-CD24 (exosomes overexpressing CD24)	Inhalation	1×10^{8} - 1×10^{10} particles per 2 ml saline	1	NCT04747574
EXO 1, EXO 2 (MSC-derived exosomes)	Inhalation	0.5-2 $\times 10^{10}$ particles in 3 ml special solution	1	NCT04491240
CSTC-Exo (COVID-19-specific T cell-derived exosomes)	Inhalation	2×10^{8} particles in 3 ml	1	NCT04389385
MSC-derived exosomes	Inhalation	2×10^{8} particles in 3 ml	1	NCT04276987

Table 2. Ongoing clinical trials evaluating efficacy of exosome therapeutics in COVID-19