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UC-MSCs Secretome Induces Proliferation of CD4+ T Cells, CD8+ T Cells, NK Cells, and Increases sPD-1 Levels in Severe COVID-19's Whole Blood

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Background: Clinical features of severe coronavirus disease 2019 (COVID-19) predominantly include respiratory symptoms and exacerbated multi-organ complications, especially in patients with comorbidities. Cellular immunity, including lymphocytes, is a critical factor in combating SARS-CoV-2 infection. However, immune dysregulation occurs in severe COVID-19 patients, characterized by cytokine storm and lymphopenia. The effectiveness of mesenchymal stem cell (MSC) therapies for COVID-19 is being assessed. The secretome released by MSC functions similarly to the cells themselves as an immunomodulator, offering potential advantages in terms of safety and cost-effectiveness. This study was conducted to assess the effect of umbilical cord MSC-derived (UC-MSC) secretome treatment on lymphocyte count and soluble programmed cell death-1 (sPD-1) levels in severe COVID-19 patient's whole blood.

Materials and methods: Twelve whole blood samples from healthy individuals and severe COVID-19 patients were analyzed for lymphocyte count and functional activation using flow cytometry, along with sPD-1 level measurement in pre-treatment and post-secretome conditions.

Results: The lymphocyte count in severe COVID-19 patients was significantly decreased, particularly for T cells and NK cells, indicating lymphopenia. Following secretome treatment, CD4+ T cell counts significantly increased compared to pre-treatment, although this change was not significant in the negative control group. Additionally, there was a minimal reduction in B cell count and an increase in sPD-1 levels. Elevated sPD-1 may alleviate T cell exhaustion by interfering with PD-1 binding to programmed death-ligand 1 (PD-L1).

Conclusion: Administration of UC-MSC secretome to the whole blood of severe COVID-19 patients suggested immune improvement, with significant increases in CD4+ T cell counts, enhanced B cell survival, and elevated sPD-1 levels.

Keywords: *COVID-19, cellular immunity, lymphocytes, secretome, MSC*

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causes coronavirus disease 2019 (COVID-19), which has rapidly escalated to an epidemic scale and poses a significant threat to human health, as declared a global pandemic by World Health Organization (WHO).[1] SARS-CoV-2 infection diminishes T cell count and function, thereby intensifying disease severity as it progresses. In severe COVID-19 patients, a decrease in total lymphocytes has been reported, with significant T cell depletion identified both in $CD4^+$ and $CD8^+$ T cell subsets.[2] Lymphopenia can indicate the severity of the illness and the prognosis for COVID-19 patients. Cytokine storm induces T cell apoptosis or necrosis, resulting in decreased lymphocyte count.[1]

COVID-19 pathogenesis has been linked with both cytokine storm and T-cell exhaustion.[3] A cytokine storm, marked by elevated pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2, IL-6, and interferon (IFN)-γ in patients' plasma/serum, and usually indicate COVID-19 disease severity.[4] In the recovery phase, there was a restoration of T cell levels and a decline in cytokine levels, indicating a negative correlation between them. T-cell exhaustion, a condition characterized by T-cell dysfunction, is commonly observed in COVID-19 patients and typically arises during persistent and chronic viral infections.[1,4] Expression levels of T-cell exhaustion marker, programmed death-1 (PD-1), on peripheral blood CD4+ and CD8+ T cell have been demonstrated to increase during COVID-19 symptomatic progression, especially in severe cases. T-cell exhaustion causes limitation of T-cell immune function and exhibits adaptive immune evasion.[1–3]

PD-1 is expressed on lymphocytes, including NK cells.[5] Binding of PD-1 to its ligand, programmed death ligand-1 (PD-L1), can inhibit immune activation by reducing proliferation, increasing apoptosis, and modulating T cell activation and effector function in cases of infection.[5,6] PD-1 is expressed in a membrane-bound and soluble form. An increase in PD-1 and soluble PD-1 (sPD-1) occurs in parallel when lymphocytes are activated.[5] sPD-1 can negatively modulate the functional interaction of PD-1/PD-L1 by blocking the binding of PD-L1 to PD-1 thereby increasing T cell function activity.[5,6]

Various treatments for COVID-19, such as drug repurposing, antivirals, convalescent plasma, neutralizing antibodies, and stem cell therapy, are under development. Mesenchymal stem cell (MSC) therapy is a potential treatment for severe COVID-19, due to its capacity to modulate immune cells, inhibit cytokine storms, and stimulate endogenous repair.[7,8] MSCs can modulate both innate and adaptive immunity, affecting various immune effectors, including neutrophils, dendritic cells (DC), macrophages, NK, B, and T cells.[9]

Trophic factors and extracellular vesicles secreted by MSCs otherwise known as "secretome", mediate intercellular signaling and tissue recovery. Recently, conventional MSCs therapy has shifted to MSC-derived-secretome based on its advantages, including without tumorigenicity, low immunogenicity, no risk of latent infection transmission, and greater ease and cost-effectiveness.[7] UC-MSC-derived-secretome contains various soluble factors in the form of growth factors, pro-angiogenic, and extracellular matrix proteins.[10] UC-MSCs-derived-secretome is considered to be a potential candidate for COVID-19 treatment due to its immunomodulatory, pro-angiogenic, anti-inflammatory, and anti-protease properties, resembling those of MSCs.[8]

The UC-MSCs secretome may represent effective therapeutic strategies for COVID-19 patients.[11] Several clinical studies found that UC-MSCs secretome improved clinical outcomes and reduced inflammatory cytokines, in severe COVID-19 patients.[12] Besides the cytokines, lymphocyte count was also the powerful parameter among biological variables for evaluating COVID-19 severity,[13] as well as post-treatment recovery progression.

This study was conducted to investigate the capacity of UC-MSCs-secretome to stimulate lymphocytes *in-vitro* proliferation, specifically $CD4^+$ T cells, $CD8^+$ T cells, B cells, and NK cells. Furthermore, sPD-1 levels were measured to evaluate lymphocyte function, as they promote activation by negatively regulating the PD-1/PD-L1 pathway. The results may be considered a potential therapeutic candidate for lymphopenia, particularly in cases of severe COVID-19.

Materials and methods

Study Subjects

The study was designed as an in-vitro experimental study using whole blood samples from a group of research subjects, which included both severe COVID-19 patients and healthy donors who met specified inclusion and exclusion criteria. The inclusion criteria for severe COVID-19 subjects were individuals aged 18-69 years who had been confirmed positive for COVID-19 through real-time reverse

transcription polymerase chain reaction (RT-PCR) from throat, sputum, or broncho-alveolar lavage (BAL) samples and were classified as severe COVID-19 patients based on the analysis by the attending physician. For healthy subjects, the inclusion criteria included individuals aged 18-69 years with negative COVID-19 confirmation through throat, sputum, or BAL swab real-time RT-PCR and no history of other infectious diseases. All study subjects provided informed consent to participate. Exclusion criteria included a medical history of allergy to penicillin, streptomycin, and amphotericin-B, current involvement in other intervention studies, and participation in other intervention studies within the preceding 3 months.

Whole blood was utilized in the study to minimize changes in the myeloid population, thereby more closely simulating in-vivo conditions, while also reducing handling time and processing time without the need for peripheral blood mononuclear cell (PBMC) isolation.[14] Hematological and flow cytometry analyses were conducted on whole blood samples obtained from both severe COVID-19 patients and healthy subjects to assess lymphocyte counts, including T, B, and NK cells. After pre-treatment analysis, both sample groups underwent intervention with the administration of 3µL of secretome, while a negative control group received no secretome intervention. Following 72 hours of incubation[15], sample suspensions were analyzed hematologically and via flow cytometry to measure lymphocyte counts under pre-treatment conditions. The culture supernatant was stored at -80°C for further measurement of soluble programmed cell death-1 (sPD-1) levels. Data obtained will be analyzed statistically using SPSS. The study was conducted at the National Central General Hospital Dr. Cipto Mangunkusumo in Jakarta for sampling severe COVID-19 subjects. Blood collection for healthy subjects, whole blood incubation with secretome intervention, routine blood tests, flow cytometry analysis for measuring lymphocyte counts (T, B, and NK cells), and sPD-1 levels were carried out at the Integrated Laboratory of the Faculty of Medicine, Universitas Indonesia.

The study included twelve whole blood samples from both healthy and severe COVID-19 subjects. The sample size for the treatment group was calculated using hypothesis testing for the mean difference of two dependent groups, pre- and post-secretome intervention in blood samples.[15] The study utilized 12 samples for each group, exceeding the minimum sample size of 11 required to detect a significant difference between the two test groups. The study was approved by the ethical committee of the Faculty of Medicine, Universitas Indonesia, with reference number KET-1451/UN2.F1/ETIK/PPM.00.02.2020. All research subjects provided informed consent to participate.

UC-MSCs-derived Secretome Preparation

The UC-MSC-derived secretome was generated and provided by the Stem Cell Medical Technology Integrated Service Unit at Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia. The secretome was produced by culturing UC-MSC in a complete medium, with periodic changes every 2-3 days. The cell culture medium containing the secretome was then collected in sterile tubes and stored at -20°C. When needed, the secretome was thawed by warming it in a water bath at 37°C.

Whole Blood Incubation with Secretome

After obtaining informed consent from the patient or their family, a medical history was taken, followed by a physical examination. A whole blood sample was then collected in a heparin tube (Cat. No. 455084, Greiner Bio-One, Bad Haller, Austria). One tube containing the blood sample was set aside for analysis of pre-intervention conditions. Each subject's blood sample was divided into two tubes. Each tube received 500µL of complete Roswell Park Memorial Institute (RPMI) medium (Cat. No. 11875093, Gibco, RPMI 1640 Medium, Thermo Fisher Scientific, NY, USA) and 500µL of whole blood. The first tube was treated with 3µL of secretome, while the second tube served as a negative control (containing only complete RPMI and blood). Both tubes were then incubated at 37°C in a 5% CO_2 environment for 72 hours, after which routine blood measurements were performed.

Lymphocyte Count Analysis

Pre-treatment blood samples were subjected to hematological analysis to assess routine blood levels, including lymphocytes, monocytes, neutrophils, basophils, and eosinophils, using an auto hematology analyzer (Biota, VABIO 580, Turkey). After 72 hours of incubation, post-intervention blood samples from both tubes (One with 3µL secretome and the other as a negative control) were also analyzed hematologically, following the same procedure used for the pre-treatment samples.

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Flow Cytometry

T, B, and NK cells were analyzed using the BD FACSCanto II flow cytometer (BD Biosciences, San Jose, USA). Lymphocyte counts were determined using the BD Multitest 6-color protocol (CD3/CD16+56/CD45/CD4/CD19/CD8) TBNK reagent (BD Biosciences, San Jose, USA, Cat No. 644611). A total of 5 µL of the BD Multitest 6-color TBNK reagent was added to the bottom of a Falcon tube (Corning Brand, BD Biosciences, Cat No. 352054), followed by the addition of 50 µL of the sample. The tube was sealed and gently mixed before incubating in the dark at room temperature for 15 minutes. Afterward, 450 µL of 1x BD FACS lysing solution (BD Biosciences, San Jose, USA, Cat No. 349202) was added, and the tube was resealed and gently mixed again. It was then incubated in the dark at room temperature for another 15 minutes. Once sample preparation was complete, the samples were analyzed using the BD FACSCanto II flow cytometer and the BD FACSCanto software. Data interpretation included total lymphocytes ($CD45^+$) and each lymphocyte subset population: T cells ($CD3^+$), $CD4^+$ T cells ($CD3^+CD4^+$), CD8+ T cells ($CD3^+CD8^+$), B cells ($CD3^-CD19^+$), and NK cells ($CD3^-CD16^+CD56^+$).

Measurement of sPD-1 levels

sPD-1 levels in culture supernatants were quantified using the Human PD-1 Quantikine ELISA Kit (Cat. No. DPD10, R&D Systems, Minneapolis, USA) according to the manufacturer's protocol. The detection range was 15.6 to 1,000 pg/mL and a sensitivity of 3.27 pg/mL. A total of 50 µL of Assay Diluent RD1-21 was added to each well, followed by 50 µL of standard solution, control, or sample. The plate was covered with an adhesive strip and incubated at room temperature for 2 hours on a microplate shaker. After incubation, the plate was washed four times with wash buffer. Next, 200 µL of Human PD-1 Conjugate was added to each well, and the plate was covered again and incubated for an additional 2 hours on a shaker. Following this, the plate was washed again, and 200 µL of substrate solution was added to each well. This was incubated at room temperature for 30 minutes, protected from light. Finally, 50 µL of stop solution was added to each well, resulting in a color change from blue to yellow. The optical density was measured using an ELISA reader (Vmax Kinetic ELISA microplate reader, Molecular Devices LLC, San Jose, USA) at 450 nm to determine the sPD-1 level (pg/mL).

Results

Subject Characteristics

The study included 12 participants each from the healthy and severe COVID-19 groups, ensuring an equal proportion of sex and age (Table 1). Severe COVID-19 patients exhibited respiratory symptoms and decreased oxygen saturation (SaO_2), necessitating respiratory support. However, clinical manifestations of COVID-19 are heterogeneous and determined by variations in immune responses.

Lymphocyte Count in Severe COVID-19 Patients were Lower than in Healthy Subjects

The results revealed a significant decrease (p=0.001) in total lymphocytes among severe COVID-19 subjects compared with healthy individuals (Table 2). The $CD4^+$ and $CD8^+$ T cells count in severe COVID-19 patients showed significant reductions (p=0.001 and p=0.028, respectively), as did NK cells (p=0.004). Although not statistically significant (p=0.232), the B cell count in severe COVID-19 patients was lower compared with healthy subjects.

UC-MSCs Secretome Increased $CD4^+$ T cells, $CD8^+$ T cells, and NK Cells Counts

The $CD4^+$ T cell count of severe COVID-19 patients in the post-secretome group (2.44±0.33 cells/µL) was significantly increased (p=0.025), while the negative control group (2.41±0.27 cells/µL) showed no significant difference compared with the pre-treatment group (2.30±0.28 cells/

Table 1. Characteristics of healthy and severe COVID-19 subjects.

Parameter	Healthy Subject	Severe COVID-19	*p*-value
Number of subjects	12	12	
Gender (Female/Male)	5/7	5/7	
Age (years)	53 (24-62)	57 (27-69)	0.369

*significant (Independent t-test, p<0.05) between two subject groups.

Table 2. Lymphocyte count in severe COVID-19 patients were lower than in healthy subjects.

Lymphocytes	Count (cell/uL)		*p*-value	Normal count (cell/µL) [16]
	Healthy Subject	Severe COVID-19		
Total ($CD45^+$)	1,578 (600-3,118.8)	618.6 (400-1,600)	0.001*	1,500 - 3,500
Subset				
$CD4^+$ T cell	485.19 (127.56-1,009.18)	197.25 (88.21-496.74)	0.001*	550 - 1,440
$CD8^+$ T cell	366.83 (104.88-1,051.77)	158.13 (62.57-425.17)	0.019*	320 - 1,250
B cell	252.06 (86.74-518.06)	172.81 (106.73-577.83)	0.232	90 - 560
NK cell	393.13 (68.98-971.37)	133.22 (42.44-250.41)	0.002*	150 - 1,100

*significant (Independent t-test, $p<0.05$) between two subject groups.

µL) (Figure 1). The $CD8^+$ T cell count of severe COVID-19 patients in the post-secretome group (306.14±209.14 cells/µL) and the negative control group (257.68±140.32 cells/µL) were significantly increased $p=0.016$) compared with the pre-treatment group(199.4±127.23 cells/µL).

In contrast, B cell counts of severe COVID-19 patients in both the negative control group ($p=0.000$) and the post-secretome group ($p=0.013$) were significantly decreased compared with the pre-treatment group (2.26 ± 0.21 cells/µL). Notably, B cell counts decreased more sharply in the negative control (1.80±0.44 cells/µL) compared with the post-secretome group (1.90±0.54 cells/µL).

NK cell counts showed no significant differences in both the post secretome group and the negative control group. However, NK cell counts in the post-secretome group (2.08±0.34 cells/µL) increased slightly compared with the pre-treatment group (2.05±0.27 cells/µL), while NK cell counts in the negative control group (2.03±0.28 cells/µL) showed a decrease.

UC-MSCs Secretome Increased sPD-1 levels

The sPD-1 levels of the healthy subject and sPD-1 levels of severe COVID-19 subjects did not show a significant difference ($p=0.576$) (Figure 2). However, the sPD-1 level in healthy subjects was higher (2.20±0.15 pg/mL) compared with the severe COVID-19 subjects (2.15±0.28 pg/mL). The sPD-1 levels of severe COVID-19 subjects in the post-secretome group (124.70 [55.38–273.38] pg/mL) significantly increased ($p=0.045$) compared with the pre-treatment group (120.60 [57.94–315.37] pg/mL), while there was no significant difference observed in negative control group (124.86[48.68-256.62] pg/mL).

Discussion

SARS-CoV-2 induces deleterious alterations in the immunological and hematological systems of infected individuals.[17] Results showed that severe COVID-19 subjects had significantly lower total lymphocytes than healthy subjects. The count of $CD4^+$ and $CD8^+$ T cells, as well as NK cells of severe COVID-19 significantly decreased compared with healthy subjects, indicating lymphopenia so as COVID-19 severity.[11] Lymphopenia may arise from the destructive impact of SARS-CoV-2 on lymphatic tissue and cytokine storm that prevents T cell proliferation and survival by triggering T cell apoptosis or necrosis.[1,17] Meanwhile, the decrease in viral load in COVID-19 is associated with NK cell activity in controlling SARS-CoV-2 replication

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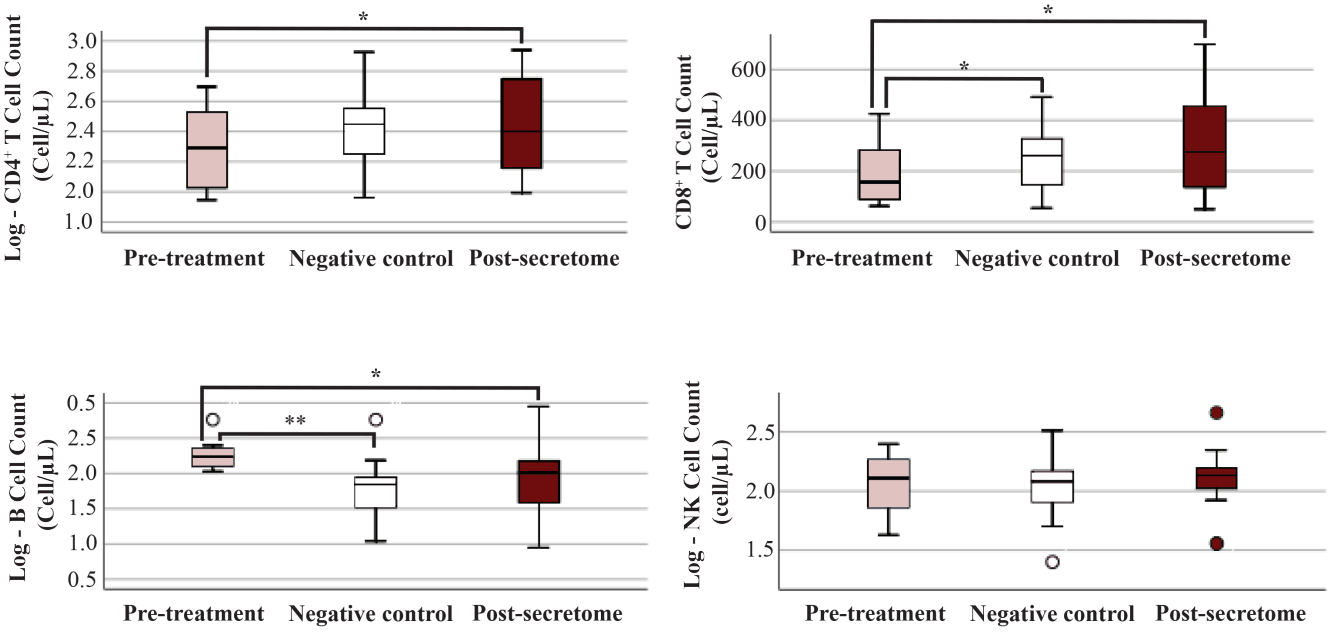

Figure 1. UC-MSCs secretome increased CD4⁺ cells, CD8⁺ T cells, and NK cells counts in severe COVID-19 patients' whole blood. *significant (Dependent t-test, $p<0.05$) compared with the pre-treatment group, **very significant (Dependent t-test, $p<0.001$) compared with the pre-treatment group.

and an increase in NK cell count.[18] In contrast, the B cell count did not show a significant difference between severe COVID-19 and healthy subjects, similar to previous study.[19] These may occur in peripheral B cells that had a shifting trend (refractory) potentially driven by T cell lymphopenia in severe COVID-19.[20] Severe infection of SARS-CoV-2 induces a strong extrafollicular B cell response but causes an impaired germinal center (GC) response.[21]

Results showed CD4⁺ and CD8⁺ T cells count significantly increased post-secretome compared with pre-treatment. The gradual increase in T cells indicated lymphocyte count was restored during recovery, a process thought to be associated with COVID-19 prognosis.[3,17,22] Restoration of cellular immunity may be a sign of the healing phase.[19] Interestingly, B cell count in severe COVID-19 subjects significantly decreases in all treatments. T cells tended to increase while B cells tended to decrease. These results align with previous study, indicating the response of T cells and B cells to SARS-CoV-2 has different kinetics.[23] Cellular immunity, as indicated by SARS-CoV-2-specific CD4⁺ and CD8⁺ T cells, was observed to be more stable and persistent than antibody-mediated humoral immunity.[19] Humoral immune response against COVID-19 is often limited durability, as observed in other human coronavirus

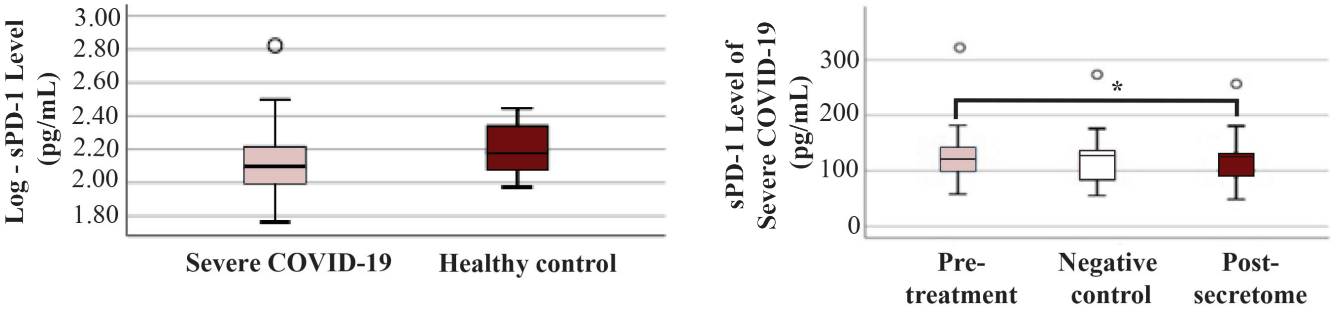

Figure 2. UC-MSCs secretome increased sPD-1 Levels in severe COVID-19 patients' whole blood. *significant (Wilcoxon test, $p<0.05$) compared with the pre-treatment group.

(huCoV) epidemics, like SARS and Middle East respiratory syndrome (MERS).[24,25] In previous study found that SARS-CoV-2 infection caused damage to germinal centres in the lymph nodes and spleen, resulting in a significant decrease in Bcl-6 + B cell counts, whereas activation-induced cytidine deaminase (AID) + B cell counts remained stable. This damage is associated with reduced durability of the humoral immune response.[24]

Interestingly, B cell count dropped significantly in the negative control and moderately in the post-secretome condition, suggesting the secretome aids in B cell survival. MSCs can modulate B cell activity through the secretion of soluble factors, include indoleamine 2,3-dioxygenase (IDO) and IL-7. When acting synergistically with IL-10, IDO produced by MSCs can promote the survival and proliferation of B cells.[26] IL-7 induces quiescent peripheral T cells to release B cell activating factor (BAFF), thus aiding in the survival of B cells.[27]

The effect of secretome is similar to MSCs in severe COVID-19 patients with lymphopenia, increasing lymphocyte count to normal levels, especially T cells. MSC immunomodulation can work through the synergistic of cell contact (paracrine) and secretome (soluble factor) mechanisms.[7,28] Soluble factors like chemokines, cytokines, and growth factors are thought to contribute to homeostasis maintenance and cell proliferation in damaged tissue.[28,29] Growth factors released by MSCs, there are vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), keratinocyte growth factor (KGF), insulin-like growth factors (IGF-1 and IGF-2), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and hepatocyte growth factor (HGF).[10,29] Growth factors drive cell proliferation by activating receptor tyrosine kinases (RTKs) and related signalling pathways, including PI3K/Akt, essential for cell cycle initiation and progression.[30]

In SARS-CoV-2 infection, MSCs can enhance T cell maturation, as evidenced by the heightened expression of early T-cell markers CD25 and CD69 observed in co-culture systems with MSCs.[26] Furthermore, RNA sequencing analyses have shown that MSCs increase the expression of genes related to inflammatory responses.[31,32] These results suggest that MSCs not only have anti-inflammatory properties but also exert immune-stimulatory effects on T lymphocytes.[26] Previous study suggested MSCs can modulate development, activation, and chemotaxis of T cells, B cells, and NK cells by upregulating specific genes, include chemotaxis and telomerase-related genes, to maintain homeostasis.[31]

A soluble form of PD-1, sPD-1, showed no significant difference between healthy and severe COVID-19 subjects, this results were in line with previous study.[33] Menwhile, sPD-1 levels in severe COVID-19 subjects increased significantly post-secretome compared to pre-treatment. Increased sPD-1 levels are linked to disease improvement. In severe COVID-19, increased sPD-1 levels reduce the immunosuppressive pathway thereby get impact on increasing T cell count and function.[34]

This study showed a significant increase in T cell count and sPD-1 levels in severe COVID-19 patients post-secretome, in line with reports that sPD-1 can induce T cell responses, both in the exhaustion phase and in primary activation of T cells.[35] MSCs possess the capability to modify different T-cell subsets, a mechanism that could potentially support recovery from SARS-CoV-2 infection.[26] UC-MSCs promote the expression of CD69, which serves as an early-stage T cell marker, and CD25, which is indicative of mid-stage T cell activation.[32] The complex processes of T-cell development, activation, and differentiation engage kinases from the mitogen-activated protein kinase (MAPK) family.[26] MSCs can act as inhibitors of extracellular-signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK), thereby supporting T-cell function by suppressing their phosphorylation.[31] MSCs can stimulate T cell function through MAPK-ERK/JNK regulation thereby promoting T cell activation and proliferation by increasing $CD28^+$ proportion on human T cell surface.[31,32] $CD28^+$ is a costimulatory receptor molecule for T cell activation. $CD28^+$ signaling activates the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of the rapamycin (mTOR) pathway thereby stimulating T-cell activation, growth, and differentiation.[36] The presence of sPD-1 can also activate the PI3K-AKT-mTOR pathway by blocking the PD-1/PD-L1 signalling pathway thereby promoting the proliferation, differentiation, and activation of T-cell function.[6,34]

The unique immunomodulatory properties of MSCs and their secretome make them potential candidates for various immune disease immunotherapy, including COVID-19. Further research and clinical investigations are necessary due to limited comprehensive data on essential roles and detailed mechanisms involved in MSCs-based therapy in the activation and immune response maintenance to SARS-CoV-2 infection, both *in vivo* and *in vitro*.[26]

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Conclusion

In vitro administration of UC-MSCs-derived-secretome to whole blood from severe COVID-19 increased the count of $CD4^+$ T cells, $CD8^+$ T cells, NK cells, and subsequently elevated sPD-1 levels, which is thought to inhibit the PD-1/PD-L1 signaling pathway.

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Authors' Contributions

HW, as the principal investigator of this study, contributed to the conception and design, funding acquisition, methodology, data validation, critically drafted the manuscript, revised the manuscript, gave final approval, and agreed to be accountable for all aspects of the work, ensuring integrity and accuracy. WS contributed to critically drafting and revising the manuscript, performing research, analyzing data, and was a major contributor to writing the manuscript. MA contributed to the conception and design, funding acquisition, resources, methodology, data validation, supervised the experiments, critically drafted the manuscript, revised the manuscript, gave final approval, and agreed to be accountable for all aspects of the work, ensuring integrity and accuracy. SP and LNS contributed to performing research, critically drafting the manuscript, and analyzing data. IKL contributed to supervising all aspects of the use of umbilical cord-mesenchymal stem cell-based secretome in this study. AB contributed to supervising and taking responsibility for all aspects of the cell culture protocol. AR contributed to supporting data interpretation and analysis. HW, WS, MA, SP, LNS, IKL, AB, and AR contributed to supporting data interpretation and analysis.

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