JOURNAL LA MEDIHEALTICO

VOL. 05, ISSUE 05 (967-977), 2024
DOI: 10.37899/journallamedihealtico.v5i5.1522

In Vitro Anti-inflammatory Effect of Secretome from Umbilical Cord-derived Mesenchymal Stem Cells in COVID-19 Patient Blood: A Study on sIL-6R, sgp130, IL-1RA and Anti/pro Inflammatory Cytokines

Nisrina Asysyifa[1], Heri Wibowo[2], Murdani Abdullah[3], Isabella Kurna Liem[4], Arleni Bustami[5]

[1]Master's Programme in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[2]Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jakarta,Indonesia
[3]Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia
[4] Departement of Anatomy, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[5]Integrated Laboratory of Medical Faculty, Universitas Indonesia, Jakarta, Indonesia

**Corresponding Author: Nisrina Asysyifa*
E-mail: asysyifan@gmail.com

Article Info

Article history:
Received 1 August 2024
Received in revised form 13 October 2024
Accepted 30 October 2024

Keywords:
COVID-19
Secretome
sil-6R
sgp130
IL-1RA

Abstract

COVID-19 exhibits a wide range of clinical manifestations which severity of the disease is linked to uncontrolled escalation of inflammatory mediators. Ongoing research has identified the immunomodulatory effects of the MSC-derived secretome as a potential therapy for COVID-19. However, the precise mechanism by which the secretome exerts its therapeutic effect on COVID-19 remains unclear. This study aims to investigate whether the components of the UC-MSC-derived secretome can alter the inflammatory characteristics of immune cells. To achieve this, an in vitro study will be conducted involving co-incubation of whole blood with secretomes, followed by LPS stimulation. A total of 12 blood samples from severe COVID-19 and healthy subjects were cultured into three groups (RPMI control, 3µl and 9µl secretome group) incubated for 24 hours. Then, the cultures were exposed to LPS for 48 hours. The levels of sIL-6R, sgp130, IL-1RA, IL-6, TNF-α, IFN-γ, and IL-10 were measured. Results showed that LPS increased IL-6, TNF-α, and IL-10 production, while reducing sIL-6R, and sgp130, but no changes seen in IFN-γ in secretome-incubated blood cultures. The post-LPS/pre-LPS ratio analysis was conducted to investigate the anti-inflammatory potential of secretome. It was found that the secretome provides its anti-inflammatory effects through the role of IL-1RA.

Introduction

The emergence of SARS-CoV-2 in late 2019 led to the COVID-19 pandemic, affecting over 184 million people and causing more than 3.98 million deaths by July 6, 2021 (NCDC, 2020). COVID-19 symptoms range from severe pneumonia and acute respiratory distress syndrome (ARDS) to mild respiratory infections or even asymptomatic cases, influenced by individual immune responses (Gao et al., 2020). Severe cases involve an inadequate immune response leading to excessive inflammation and a cytokine storm, primarily driven by increased IL-6 levels, which correlate with disease severity (Shekhawat et al., 2021).

IL-6, a multifunctional cytokine, regulates immune responses, hematopoiesis, acute phase reactions, and inflammation. IL-6 interacts with its receptors (IL-6R and gp130), activating signaling pathways that can be pro-inflammatory or anti-inflammatory (Mihara et al., 2011). The body has a mechanism involving soluble gp130 (sgp130) to antagonize IL-6/sIL-6R

complexes, stabilizing the inflammatory response (Dinarello, 2018). When IL-6 binds to membrane-bound IL-6R (mIL-6R), it forms a high-affinity functional receptor complex with gp130. Soluble IL-6R (sIL-6R) can also interact with IL-6, forming a complex that subsequently associates with gp130. This receptor complex homodimerization activates Janus kinases (JAKs), which then phosphorylate tyrosine residues within the cytoplasmic domain of gp130. The activation of JAKs mediated by gp130 through IL-6 initiates two principal signaling pathways: the SHP-2 pathway, derived from Tyr759 of gp130 and associated with the MAPK ERK pathway, and the gp130 YXXQ-mediated JAK/STAT pathway (Mihara et al., 2011). Signaling through the mIL-6R pathway generally conveys anti-inflammatory signals, while signaling through the sIL-6R pathway tends to promote pro-inflammatory responses, reflecting the pleiotropic nature of IL-6.

The body has a regulatory mechanism involving sgp130, which arises from alternative splicing of gp130 mRNA. sgp130 acts antagonistically by sequestering the IL-6/sIL-6R complex in the bloodstream ((Tanaka et al., 2018). The anti-inflammatory actions of IL-6, due to its pleiotropic effects, include the suppression of pro-inflammatory cytokines such as IL-1, TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF), and the induction of IL-10, IL-1RA, and soluble TNF-α receptors. IL-10, in particular, limits CD4+ T cell activation by promoting the expansion of regulatory T cells (Tregs) (Dinarello, 2018; Turner et al., 2014).

For severe COVID-19, antiviral drugs and oxygen therapy are used, but immunomodulatory therapies like glucocorticoids, convalescent plasma, and anti-IL-6 receptor antibodies have potential benefits (Richardson et al., 2020). Mesenchymal stem cell (MSC)-based immunomodulatory therapy shows promise, with MSCs reducing inflammation and promoting tissue regeneration. MSC secretomes, rich in bioactive factors, can modulate immune responses and enhance healing (Shi et al., 2021). The immunomodulatory effects of MSC secretomes, especially from umbilical cord-derived MSCs (UC-MSC), are being studied for their potential in treating severe COVID-19 (Chouw et al., 2022). This research investigates the anti-inflammatory potential of UC-MSC-derived secretome by analyzing changes in pro- and anti-inflammatory biomarkers in blood exposed to lipopolysaccharides (LPS), a strong inflammatory agent.

Methods

This study is an experimental investigation involving the collection of fresh whole blood from severely ill COVID-19 patients who meet specific inclusion and exclusion criteria. The Integrated Medical Technology Service Unit at the Stem Cell Research Center, RSCM-FKUI, produced and supplied the UC-MSC-derived secretome for this study. To culture 125,000 MSC-UC cells, Minimum Essential Medium alpha (aMEM) was used, supplemented with 1% streptomycin-penicillin, 10% fetal bovine serum (FBS), Glutamax, and 1% amphotericin. The cells were grown in T-25 cm^2 Corning flasks at 37°C in a 5% CO_2 and 95% O_2 environment. The medium was replaced every 2-3 days, and once cells reached 80-90% confluence, they were harvested, washed with 1x Phosphate Buffered Saline (PBS), and incubated in serum-free aMEM for 24 hours to collect the conditioned medium containing the UC-MSC-derived secretome. This medium was centrifuged at 3500 rpm for 30 minutes, filtered through 0.45 µm and 0.22 µm filters, and diluted with high-glucose Dulbecco's Modified Eagle Medium (DMEM) to 50% concentration. The resulting secretome was stored at -20°C in sterile tubes, underwent rigorous quality control including sterilization and protein content analysis, and was thawed in a 37°C water bath prior to use. Characterization of MSC-UC and the secretome was also performed.

Blood samples from each subject will be divided into three sets of two tubes each: one set for healthy controls and one for COVID-19 patients. To prevent aerosol contamination, screw-cap tubes will be used. Each tube will contain 500 µL of complete Roswell Park Memorial Institute

(RPMI) medium and 500 µL of whole blood. For COVID-19 patient blood cultures, the secretome will be used at volumes reflecting physiological and elevated IL-6 levels. Specifically, 3 µL of secretome will yield an IL-6 concentration of 8.7 pg/3 µL, and 9 µL will produce an IL-6 concentration of 26.1 pg/3 µL. The first set of tubes will receive 3 µL of secretome, the second set 9 µL, and a control tube will contain only RPMI medium and whole blood without secretome. The tubes will be incubated at 37°C in a humidified atmosphere with 5% CO_2 for 24 hours, with caps loosely secured and tubes maintained upright. After incubation, cell pellets will be collected and supernatants will be harvested. The cell pellets will be resuspended in 10 µL of LPS (10,000 ng/mL) to achieve a final LPS concentration of 100 ng/mL. RPMI medium will be added to reach a final volume of 990 µL in each tube. The resuspended pellets will be incubated for an additional 48 hours under the same conditions. Following this, supernatants will be harvested, aliquoted, and stored at -80°C until analysis.

This study quantifies the levels of sIL-6R, sgp130, IL-1RA, and the mediators IFN-γ, IL-6, TNF-α, and IL-10 in stock secretome and supernatant samples from each treatment group. Measurements are conducted using a magnetic Luminex multiplex immunoassay with human premixed multi-analyte kits from R&D Systems: lot number 1689431 for sIL-6R and sgp130, and lot number 895546 for IFN-γ, IL-6, TNF-α, IL-10, and IL-1RA, following the manufacturer's instructions. Data analysis is performed using SPSS software, version 23. Both univariate and bivariate analyses are applied to the parameters sIL-6R, sgp130, IL-1RA, IFN-γ, IL-6, TNF-α, and IL-10. Statistical significance is determined with a 95% confidence level ($\alpha = 0.05$).

Results and Discussion

This study aimed to assess the anti-inflammatory effects of blood cell culture components from COVID-19 patients after incubation with UC-MSC-derived secretome and subsequent LPS exposure. Due to the high IL-6 levels in the secretome (2995.63 pg/mL), an in vitro approach was chosen to avoid the risks associated with IL-6's pro-inflammatory effects in vivo. The study used 3 µL of secretome to achieve a physiological IL-6 level of approximately 12 pg/mL (with 8.7 pg of IL-6), and 9 µL to induce a significantly elevated IL-6 level (26.1 pg/3 µL) for inflammatory response.

Cell counts were not performed for each subject. To reduce bias, comparisons were made within each group before and after LPS exposure, rather than between different subject groups. This approach ensured that changes in analyte levels reflected cellular responses accurately. The study evaluated how LPS exposure influenced the secretion of pro-inflammatory and anti-inflammatory mediators in blood cultures from COVID-19 patients treated with the secretome. Differences in mediator production were compared to conditions without secretome (RPMI control) and expressed as ratios relative to LPS exposure.

Twelve individuals with severe COVID-19, who had not yet received anti-IL-6 therapy, participated in the study. The median age of these participants was 57 years (range: 27-69), with a gender distribution of 5 females and 7 males. Seven were non-smokers and three were former smokers. The participants were treated in various settings: one in the emergency department (ED), two in the intensive care unit (ICU) with mechanical ventilation support, and five in the High Care Unit (HCU). Each participant had at least one comorbidity, ranging up to five, including renal disease, hypertension, diabetes mellitus, stroke, and obesity. The study also included twelve healthy control subjects, confirmed negative for COVID-19, with no history of pulmonary infections and who were non-smokers. The median age of the controls was 53 years (range: 24-62), with a gender distribution of 5 females and 7 males.

The outcomes of the preliminary assessments of soluble mediators and receptors in individuals with COVID-19 and healthy controls are summarized in Table 1. These measurements were taken both at baseline (prior to secretome stimulation) and following secretome intervention.

Table 1. Concentrations of Soluble Mediators and Receptors in COVID-19 Patients and Healthy Controls Prior to LPS Exposure

Parameter (pg/mL)	Intervention	COVID-19 Subject	Healthy Subject	*p value*
sIL-6R	Baseline	22.380,35	13.674,95	.000*
	24h S3	18.866,96	13.431,27	.001*
	24h S9	18.497,87	13.322,95	.002*
sgp130	Baseline	49.317,10	51.081,02	.826
	24h S3	55.937,51	52.556,51	.372
	24h S9	58.880,41	53.173	.149
IL-1RA	Baseline	3.277,68	2.409,58	.336
	24h S3	4.297,06	3791,83	.418
	24h S9	3.473,57	4044,14	.368
IL-6	Baseline	4,58	4,14	.429
	24h S3	37,24	30,12	.001*
	24h S9	44,72	66,62	.241
TNF-α	Baseline	5,69	5,57	.869
	24h S3	4,70	5,22	.236
	24h S9	4,51	5,45	.082
IFN-γ	Baseline	20,02	25,51	.004*
	24h S3	20,73	28,82	.000*
	24h S9	20,53	22,78	.138
IL-10	Baseline	2,83	2,50	.521
	24h S3	4,01	2,93	.013*
	24h S9	3,25	3,69	.622

Data are presented as medians.

*p-value <0.005 (indicating a significant difference).

Note: Baseline = initial condition before incubation; 24h S3 = 24-hour incubation with 3 µL secretome intervention; 24h S9 = 24-hour incubation with 9 µL secretome intervention.

Initial measurements indicate significantly elevated sIL-6R levels in COVID-19 patients compared to healthy controls. A study also reported higher levels of sIL-6R, IL-6, and TNF-α in COVID-19 patients, with reduced sgp130 levels (Di Spigna et al., 2020). IFN-γ levels are markedly lower in severe COVID-19 cases, as supported by Giamarellos-Bourboulis et al. (2020) suggesting that IFN-γ may not be a direct driver of hyperinflammation in severe cases (Chen et al., 2020).

The reduction in IFN-γ is likely associated with CD4+ and CD8+ T cell exhaustion, as evidenced by decreased T cell counts and elevated PD-1 expression in ICU-admitted patients (Diao et al., 2020). Exhausted CD8+ T cells exhibit diminished effector functions, resulting in lower IFN-γ and IL-2 production, which also impairs Th1 cell polarization. Despite these changes, IL-1RA and IL-10 levels in COVID-19 patients are not significantly different from those in healthy controls, although they are somewhat elevated.

Blood cultures from COVID-19 patients exposed to UC-MSC-derived secretome in vitro demonstrated significantly elevated sIL-6R levels compared to healthy controls, with notable differences at both 3 µL and 9 µL secretome concentrations. IL-6 levels were also higher in COVID-19 patients at the 3 µL secretome concentration. Xu et al. (2022) observed similar increases in IL-6 with UC-MSC-derived secretome, followed by a subsequent reduction in IL-

6 levels, suggesting that UC-MSC-derived secretome therapy might be effective in managing elevated inflammatory cytokines.

IFN-γ levels were significantly lower in the COVID-19 group treated with 3 µL of secretome, indicating a potential therapeutic benefit in modulating excessive inflammation and supporting immune balance. This decrease in IFN-γ may reflect infection resolution and immune system normalization, as supported by Channappanavar et al. (2019) and Negishi et al. (2018). One study reported significant reductions in IL-1RA, IFN-γ, and TNF-α levels in COVID-19 patients receiving UC-MSC therapy (Meng et al., 2020). Similarly, Hashemian et al. (2021) found decreased TNF-α and IL-8 levels in patients with ARDS due to COVID-19 following UC-MSC treatment, with some reduction in IFN-γ.

IL-10 levels were significantly higher in the COVID-19 group treated with 3 µL of secretome, suggesting that the therapy might be fostering an anti-inflammatory response and aiding in inflammation resolution. Studies indicate that MSC can shift pro-inflammatory M1 macrophages to an anti-inflammatory M2 phenotype, accompanied by increased IL-10 production. This reprogramming is influenced by conditioned MSC media and factors such as IGF-1 and Ang-1, which have anti-inflammatory effects and support immune modulation (Tang et al., 2017). Meanwhile, levels of sgp130, IL-1RA, and TNF-α did not show significant differences between COVID-19 patients and healthy controls with 3 µL and 9 µL secretome treatments.

LPS is frequently employed in scientific research to investigate inflammatory mechanisms due to its ability to induce various inflammatory responses, including the release of pro-inflammatory mediators such as IL-6, IL-1β, and TNF-α. The inflammatory response is initiated through Toll-like receptor (TLR) signaling, which involves adaptor proteins and the activation of NF-κB and AP-1 pathways (Kang et al., 2021). TLR4, the primary receptor for LPS, triggers the recruitment and activation of MyD88, IL-1R kinase (IRAK), TNF receptor-associated factor 6 (TRAF-6), and NADPH oxidase (Nox), leading to NF-κB activation (Ngkelo et al., 2012).

Changes in sIL-6R, sgp130, and IL-1RA profiles following LPS exposure were analyzed (Figure 1). The analysis involved a 48-hour incubation with LPS across three treatment groups: control RPMI, 3 µL secretome, and 9 µL secretome. The levels of sIL-6R were assessed before and after LPS exposure in each group. The ratios of mediator production between the RPMI control (without secretome) and the secretome-treated groups (3 µL and 9 µL) were compared to understand how the secretome affects inflammation. The expression of each analyte was evaluated by comparing the post-LPS stimulation levels to the pre-LPS stimulation levels.

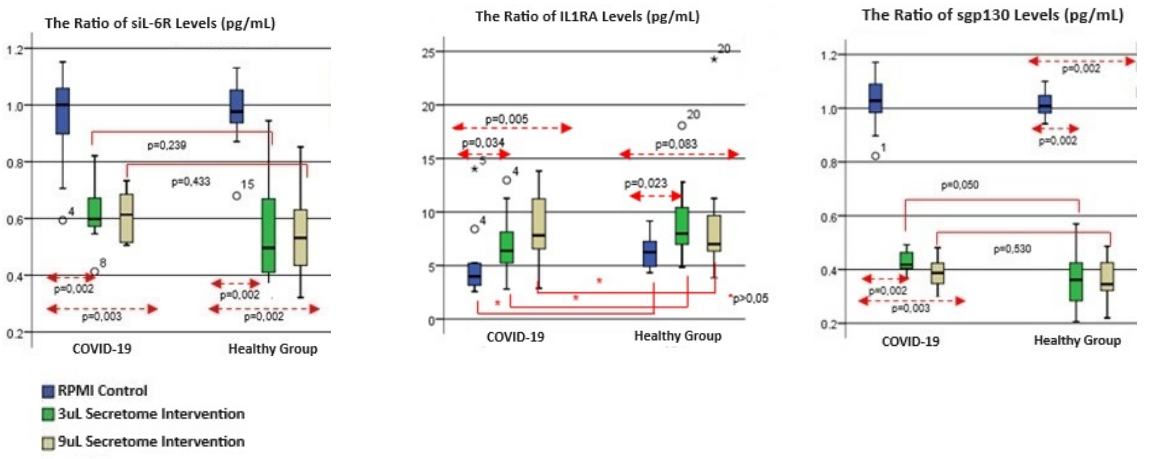

Figure 1. Effect of Secretome Incubation on a) siL-6R, b) IL1RA, c) sgp130 in total blood cultures with LPS stimulation (pg/mL)

In the RPMI control group, levels of sIL-6R and sgp130 did not significantly change after LPS exposure in either COVID-19 or healthy subjects. Although LPS typically induces an increase in sIL-6R and sgp130 levels, no substantial rise was observed. sIL-6R, a soluble form of the membrane-bound IL-6 receptor, is cleaved from the cell membrane by the protein ADAM17, which is activated under pro-inflammatory conditions (Rose-John, 2020) (Memoli et al., 2005). Similarly, sgp130, a soluble form of gp130, is generated through ADAM17 activity and alternative splicing of gp130 mRNA (Vermes et al., 2002). The absence of increased sIL-6R production in the RPMI control group following LPS exposure may be attributed to inadequate activation of the adaptive immune system, including B and T cells.

Intriguingly, the treatment groups exposed to LPS demonstrated a significant decrease in sIL-6R levels. One hypothesis for this reduction is that IL-6R production is inhibited, or there is an impediment in releasing IL-6R from its transmembrane form, resulting in decreased sIL-6R concentrations. Additionally, it appears that the secretome might inhibit sIL-6R protein synthesis or reduce ADAM10/17 activity.

A significant decrease in sgp130 production was also observed in the LPS-stimulated treatment groups in both COVID-19 and healthy subjects. This finding is interesting because sIL-6R and sgp130 are inversely correlated in IL-6 signaling. While sIL-6R promotes trans IL-6 signaling, sgp130 acts as an inhibitor by binding to the IL-6/sIL-6R complex and blocking downstream signaling. This suggests that the role of sgp130 as a co-receptor for sIL-6R is not functioning correctly, leading to inhibited sgp130 synthesis or reduced ADAM17 activity. However, this inhibitory mechanism warrants further investigation.

In COVID-19 subjects, UC-MSC-derived secretome significantly increased IL-1RA levels in response to LPS, with the 9 µL secretome group showing the greatest increase compared to the control and 3 µL secretome groups. Notably, the 9 µL secretome also had higher IL-6 levels than the 3 µL secretome. However, in healthy subjects, no significant difference in IL-1RA levels was observed between the RPMI control and the 9 µL secretome group. This may be due to lower baseline IL-1RA levels in healthy subjects and potential inhibition of IL-1RA production by LPS and the 9 µL secretome. IL-6, which is elevated in the 9 µL secretome group, is known to enhance IL-1RA and IL-10 production, contributing to an anti-inflammatory response and helping to regulate inflammation and maintain immune homeostasis (Steensberg et al., 2003).

Then, the production of pro-inflammatory and anti-inflammatory mediators in complete blood cultures from subjects exposed to LPS was quantified. Pro-inflammatory mediators, including IL-6, TNF-α, and IFN-γ, are depicted in Figures 2a-2c, while the anti-inflammatory mediator IL-10 is shown in Figure 2d. Result from this study demonstrated that LPS administration stimulates immune cells in blood cultures, resulting in significant increases in the levels of IL-1RA, IL-6, TNF-α, and IL-10. This suggests that LPS exposure activates specific cellular signaling pathways in immune cells, resulting in elevated production of these inflammatory mediators.

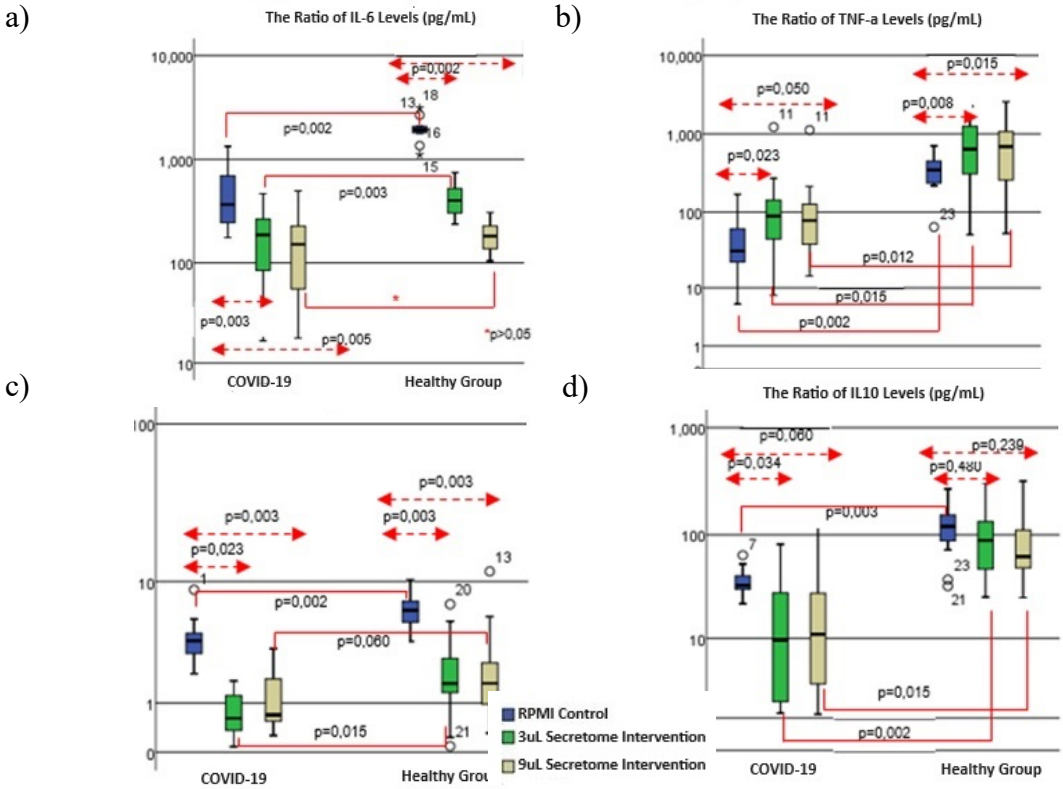

Figure 2. Effect of Secretome Incubation on a) IL-6, b) TNF-α, c) IFN-γ, d) IL-10 in Total Blood Cultures with LPS Stimulation (pg/mL)

Analysis of the post-LPS/pre-LPS ratio revealed an increase in IL-6 in both COVID-19 and healthy subjects, though this increase was less pronounced with secretome treatment. This was further supported by observing a significant reduction in IL-6 in the secretome-treated group compared to the control, suggesting that the secretome may inhibit IL-6 synthesis or reduce TLR expression. The rise in IL-6 following LPS stimulation is thought to contribute to an increase in IL-1RA, as previous studies show that IL-6 exposure enhances IL-1RA production in macrophages (Tilg et al., 1994).

The UC-MSC-derived secretome demonstrated limited efficacy in inhibiting TNF-α, with higher TNF-α levels observed in the control group compared to the secretome-treated group. Although a significant increase in TNF-α was noted in healthy subjects, it remains unclear if the secretome has a more pronounced anti-inflammatory effect in COVID-19 patients. Meanhile, IFN-γ levels showed no significant change in COVID-19 subjects post-LPS stimulation, while healthy subjects in the 3 µL secretome group exhibited an increase in IFN-γ. Both subject groups in the RPMI control experienced a significant rise in IFN-γ following LPS exposure. This is consistent with findings from Y. Xu et al. (2021), which reported higher IFN-γ concentrations in LPS-induced ALI mouse models' control groups compared to MSC-treated groups, suggesting an immunosuppressive effect of MSCs. This effect is associated with the suppression of Th1 and Th17 responses through inhibition of T-bet and RORγT transcription factors and reduced IL-17 secretion (Volarevic et al., 2011).

In both subject groups, analysis of the post-LPS/pre-LPS ratio revealed a significant decrease in IFN-γ in the secretome-treated group compared to the RPMI group without secretome. This reduction in IFN-γ is potentially attributed to disruptions in Th1 cells affecting IFN-γ production (Campbell et al., 2014). Tau et al. (2001) showed that differential expression of cytokine receptors like IFN-γR2 can influence Th cell responses, with sustained IFN-γR2 expression inhibiting Th1 differentiation (Dobashi et al., 2001).

ISSN 2721-1215 (Print), ISSN 2721-1231 (Online)

For IL-10, the post-LPS/pre-LPS ratio analysis demonstrated a significant decrease between the RPMI control and the 3 µL secretome group, but not between the RPMI and 9 µL secretome groups. Incubation with secretome followed by LPS exposure led to a ten-fold increase in IL-10 compared to pre-LPS levels, though this increase was less pronounced than the nearly fifty-fold rise observed in cultures without secretome. In healthy subjects, no significant differences were observed among treatment groups post-LPS exposure, suggesting that while the secretome may modulate IL-10 levels, further research is necessary to elucidate the underlying mechanisms.

The research suggests that the anti-inflammatory effects of the UC-MSC-derived secretome may be primarily mediated through IL-1RA rather than IL-10. Unexpectedly, IL-10 levels did not increase as anticipated in the COVID-19 group but were higher in the RPMI control group without secretome treatment. The significant reduction in sIL-6R levels is noteworthy because high sIL-6R levels can promote anti-inflammatory effects by forming complexes with sgp130. A decrease in sIL-6R could diminish these effects. mIL-6R levels are crucial for IL-6's anti-inflammatory action through its complexes with mIL-6R. ADAM10/17, which affects the release of IL-6R and gp130 from the membrane, is also important. The decrease in sgp130 levels, which balances excess IL-6/sIL-6R complexes, may indicate that mIL-6R levels are already high, reducing the need for sgp130.

The reduction in IL-6 levels in the RPMI control group aligns with the decreased sIL-6R levels, while IL-1RA levels increased notably. However, IL-1RA levels were not higher in the secretome-treated groups compared to controls, suggesting that the increase in IL-1RA might inhibit sIL-6R production or that a promoter for the sIL-6R gene may be inactive. Further investigation is needed to understand these mechanisms.

Conclusion

The study's findings indicate that LPS exposure to whole blood cultures of COVID-19 patients that have been incubated with UC-MSC-derived secretome reduces the production of sIL-6R, sgp130, IL-10, IL-1RA, and IL-6 but does not alter the production of TNF-α and IFN-□. Consequently, IL-1RA is responsible for the UC-MSC-derived secretome anti-inflammatory capacity in COVID-19 patient blood cultures.

Further studies are needed to clarify the expression profiles of ADAM 10 and ADAM 17 to identify factors influencing soluble receptor production. Research should also investigate the role of micro-RNAs as modulators of pro-inflammatory and anti-inflammatory cytokines. Additionally, examining membrane-bound IL-6R (mIL-6R) expression is crucial for understanding the diverse effects of IL-6. Comprehensive studies should also identify specific Toll-like Receptors (TLRs) to better understand the inflammatory mechanisms triggered by secretome exposure and LPS stimulation in cultured cells.

Acknowledgment

The Ministry of Research and Technology of the Republic of Indonesia / National Research and Innovation Agency provided funding for this research.

References

Campbell, B. M., Charych, E., Lee, A. W., & Möller, T. (2014). Kynurenines in CNS disease: regulation by inflammatory cytokines. *Frontiers in Neuroscience*, *8*, 12. https://doi.org/10.3389/fnins.2014.00012

Channappanavar, R., Fehr, A. R., Zheng, J., Wohlford-Lenane, C., Abrahante, J. E., Mack, M., Sompallae, R., McCray, P. B. J., Meyerholz, D. K., & Perlman, S. (2019). IFN-I response timing relative to virus replication determines MERS coronavirus infection

outcomes. *The Journal of Clinical Investigation*, *129*(9), 3625–3639. https://doi.org/10.1172/JCI126363

Chen, Y., Liu, Q., & Guo, D. (2020). Emerging coronaviruses: Genome structure, replication, and pathogenesis. *Journal of Medical Virology*, *92*(4), 418–423. https://doi.org/10.1002/jmv.25681

Chouw, A., Milanda, T., Sartika, C. R., Kirana, M. N., Halim, D., & Faried, A. (2022). Potency of Mesenchymal Stem Cell and Its Secretome in Treating COVID-19. *Regenerative Engineering and Translational Medicine*, *8*(1), 43–54. https://doi.org/10.1007/s40883-021-00202-5

Di Spigna, G., Spalletti Cernia, D., Vargas, M., Buonavolontà, L., Servillo, G., & Postiglione, L. (2020). Drastically elevated levels of Interleukin-6 and its soluble receptor complex in COVID-19 patients with acute respiratory distress. *Clinical and Medical Investigations*, *5*(3), 1–4. https://doi.org/10.15761/cmi.1000211

Diao, B., Wang, C., Tan, Y., Chen, X., Liu, Y., Ning, L., Chen, L., Li, M., Liu, Y., Wang, G., Yuan, Z., Feng, Z., Zhang, Y., Wu, Y., & Chen, Y. (2020). Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019 (COVID-19). *Frontiers in Immunology*, *11*, 827. https://doi.org/10.3389/fimmu.2020.00827

Dinarello, C. A. (2018). Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunological Medicine*, *281*(1), 8–27. https://doi.org/10.1111/imr.12621.Overview

Dobashi, K., Aihara, M., Araki, T., Shimizu, Y., Utsugi, M., Iizuka, K., Murata, Y., Hamuro, J., Nakazawa, T., & Mori, M. (2001). Regulation of LPS induced IL-12 production by IFN-gamma and IL-4 through intracellular glutathione status in human alveolar macrophages. *Clinical and Experimental Immunology*, *124*(2), 290–296. https://doi.org/10.1046/j.1365-2249.2001.01535.x

Gao, Z., Xu, Y., Sun, C., Wang, X., Guo, Y., & Qiu, S. (2020). A systematic review of asymptomatic infections. *Journal of Microbiology Immunology and Infection*, *51*(1), 12–16.

Giamarellos-Bourboulis, E. J., Netea, M. G., Rovina, N., Akinosoglou, K., Antoniadou, A., Antonakos, N., Damoraki, G., Gkavogianni, T., Adami, M.-E., Katsaounou, P., Ntaganou, M., Kyriakopoulou, M., Dimopoulos, G., Koutsodimitropoulos, I., Velissaris, D., Koufargyris, P., Karageorgos, A., Katrini, K., Lekakis, V., … Koutsoukou, A. (2020). Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure. *Cell Host & Microbe*, *27*(6), 992-1000.e3. https://doi.org/https://doi.org/10.1016/j.chom.2020.04.009

Hashemian, S.-M. R., Aliannejad, R., Zarrabi, M., Soleimani, M., Vosough, M., Hosseini, S.-E., Hossieni, H., Keshel, S. H., Naderpour, Z., Hajizadeh-Saffar, E., Shajareh, E., Jamaati, H., Soufi-Zomorrod, M., Khavandgar, N., Alemi, H., Karimi, A., Pak, N., Rouzbahani, N. H., Nouri, M., … Baharvand, H. (2021). Mesenchymal stem cells derived from perinatal tissues for treatment of critically ill COVID-19-induced ARDS patients: a case series. *Stem Cell Research & Therapy*, *12*(1), 91. https://doi.org/10.1186/s13287-021-02165-4

Kang, D. Y., Sp, N., Jo, E. S., Rugamba, A., Kim, H. Do, Kim, I. H., Park, J.-C., Bae, S. W., Jang, K.-J., & Yang, Y. M. (2021). Non-toxic sulfur inhibits LPS-induced inflammation by regulating TLR-4 and JAK2/STAT3 through IL-6 signaling. *Molecular Medicine Reports*, *24*(1). https://doi.org/10.3892/mmr.2021.12124

Memoli, B., Grandaliano, G., Soccio, M., Postiglione, L., Guida, B., Bisesti, V., Esposito, P., Procino, A., Marrone, D., Michael, A., Andreucci, M., Schena, F. P., & Pertosa, G. (2005). In Vivo Modulation of Soluble "Antagonistic" IL-6 Receptor Synthesis and Release in ESRD. *Journal of the American Society of Nephrology*, *16*(4).

Meng, F., Xu, R., Wang, S., Xu, Z., Zhang, C., Li, Y., Yang, T., Shi, L., Fu, J., Jiang, T., Huang, L., Zhao, P., Yuan, X., Fan, X., Zhang, J. Y., Song, J., Zhang, D., Jiao, Y., Liu, L., … Wang, F. S. (2020). Human umbilical cord-derived mesenchymal stem cell therapy in patients with COVID-19: a phase 1 clinical trial. *Signal Transduction and Targeted Therapy*, *5*(1). https://doi.org/10.1038/s41392-020-00286-5

Mihara, M., Hashizume, M., Yoshida, H., Suzuki, M., & Shiina, M. (2011). IL-6/IL-6 receptor system and its role in physiological and pathological conditions. *Clinical Science*, *122*(4), 143–159. https://doi.org/10.1042/CS20110340

NCDC. (2020). *Coronavirus disease (COVID-2019) situation reports*. World Health Organisation.

Negishi, H., Taniguchi, T., & Yanai, H. (2018). The Interferon (IFN) Class of Cytokines and the IFN Regulatory Factor (IRF) Transcription Factor Family. *Cold Spring Harbor Perspectives in Biology*, *10*(11). https://doi.org/10.1101/cshperspect.a028423

Ngkelo, A., Meja, K., Yeadon, M., Adcock, I., & Kirkham, P. A. (2012). LPS induced inflammatory responses in human peripheral blood mononuclear cells is mediated through NOX4 and Giα dependent PI-3kinase signalling. *Journal of Inflammation (London, England)*, *9*(1), 1. https://doi.org/10.1186/1476-9255-9-1

Richardson, S., Hirsch, J. S., Narasimhan, M., Crawford, J. M., McGinn, T., Davidson, K. W., & Consortium, and the N. C.-19 R. (2020). Presenting Characteristics, Comorbidities, and Outcomes Among 5700 Patients Hospitalized With COVID-19 in the New York City Area. *JAMA*, *323*(20), 2052–2059. https://doi.org/10.1001/jama.2020.6775

Rose-John, S. (2020). Interleukin-6 signalling in health and disease. *F1000 Research*, *9*(Faculty Rev), 1013. https://doi.org/10.12688/f1000research.26058.1

Shekhawat, J., Gauba, K., Gupta, S., Purohit, P., Mitra, P., Garg, M., Misra, S., Sharma, P., & Banerjee, M. (2021). Interleukin-6 Perpetrator of the COVID-19 Cytokine Storm. *Indian Journal of Clinical Biochemistry*, *36*(4), 440–450. https://doi.org/10.1007/s12291-021-00989-8

Shi, L., Wang, L., Xu, R., Zhang, C., Xie, Y., Liu, K., Li, T., Hu, W., Zhen, C., & Wang, F. S. (2021). Mesenchymal stem cell therapy for severe COVID-19. *Signal Transduction and Targeted Therapy*, *6*(1), 1–5. https://doi.org/10.1038/s41392-021-00754-6

Steensberg, A., Fischer, C. P., Keller, C., Møller, K., & Pedersen, B. K. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. *American Journal of Physiology. Endocrinology and Metabolism*, *285*(2), E433-7. https://doi.org/10.1152/ajpendo.00074.2003

Tanaka, T., Narazaki, M., & Kishimoto, T. (2018). Interleukin (IL-6) Immunotherapy. *Cold Spring Harbor Perspectives in Biology*, *10*, 1–15. http://doi.org/10.1101/cshperspect.a028456

Tang, X.-D., Shi, L., Monsel, A., Li, X.-Y., Zhu, H.-L., Zhu, Y.-G., & Qu, J.-M. (2017). Mesenchymal Stem Cell Microvesicles Attenuate Acute Lung Injury in Mice Partly Mediated by Ang-1 mRNA. *Stem Cells (Dayton, Ohio)*, *35*(7), 1849–1859. https://doi.org/10.1002/stem.2619

Tau, G. Z., Cowan, S. N., Weisburg, J., Braunstein, N. S., & Rothman, P. B. (2001). Regulation of IFN-γ signaling is essential for the cytotoxic activity of CD8+ T cells. *The Journal of Immunology*, *167*(10), 5574–5582.

Tilg, H., Trehu, E., Atkins, M. B., Dinarello, C. A., & Mier, J. W. (1994). Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. *Blood*, *83*(1), 113–118.

Turner, M. D., Nedjai, B., Hurst, T., & Pennington, D. J. (2014). Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochimica et Biophysica Acta - Molecular Cell Research*, *1843*(11), 2563–2582. https://doi.org/10.1016/j.bbamcr.2014.05.014

Vermes, C., Jacobs, J. J., Zhang, J., Firneisz, G., Roebuck, K. A., & Glant, T. T. (2002). Shedding of the interleukin-6 (IL-6) receptor (gp80) determines the ability of IL-6 to induce gp130 phosphorylation in human osteoblasts. *The Journal of Biological Chemistry*, *277*(19), 16879–16887. https://doi.org/10.1074/jbc.M200546200

Volarevic, V., Arsenijevic, N., Lukic, M. L., & Stojkovic, M. (2011). Concise review: Mesenchymal stem cell treatment of the complications of diabetes mellitus. *Stem Cells (Dayton, Ohio)*, *29*(1), 5–10. https://doi.org/10.1002/stem.556

Xu, R., Feng, Z., & Wang, F. (2022). Mesenchymal stem cell treatment for COVID-19. *EBiomedicine*, *77*(Figure 1). https://doi.org/10.1016/j.ebiom.2022.103920

Xu, Y., Zhu, J., Feng, B., Lin, F., Zhou, J., Liu, J., Shi, X., Lu, X., Pan, Q., Yu, J., Zhang, Y., Li, L., & Cao, H. (2021). Immunosuppressive effect of mesenchymal stem cells on lung and gut CD8+ T cells in lipopolysaccharide-induced acute lung injury in mice. *Cell Proliferation*, *54*(5), e13028. https://doi.org/https://doi.org/10.1111/cpr.13028