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The Potency of Hematopoietic Stem Cells (HSCs) and Natural Killer (NK) Cells as A Therapeutic of SARS-CoV-2 Indonesia Isolates Infection by Viral Inactivation (In Vitro Study)

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ABSTRACT

Background: The prevalence of COVID-19 cases in Indonesia as of June 9, 2020, has been confirmed 32.076 positive cases, with 1.923 death cases. The total number of deaths reached 92,941 cases. There has been a recent update on stem cell-based biological, medical therapy as an optional treatment to handling COVID-19 due to its potential viability besides using the prevalent conventional chemical drug therapy.
Methods: In this study, in vitro research was conducted to determine the potential of hematopoietic stem cells (HSCs) and natural killer cells (NK cells) against SARS-CoV-2 viruses, which virus isolates were collected in Indonesia. The SARS-CoV-2 virus was planted in rat kidney cells and Vero cells. The cells that had been planted with the virus were given HSCs and NK cells, followed by being evaluated at intervals of 24, 48, and 72 hours. The evaluation was done by collecting cells and supernatant from the cell plate and then determining the viral load using a Polymerase Chain Reaction (PCR) machine.
Results: The results showed that the addition of HSCs and NK on cells that had been infected by SARS-CoV-2 resulted in a decrease in viral load within 24 to 72 hours in all variations of Multiples of Infection (MoI) values.
Conclusions: The administration of HSCs and NK cells has the potential to eliminate the SARS-CoV-2 virus. Although this study is only an in vitro study, it could be the basis for the development of alternative stem cell-based therapies to tackle COVID-19 cases.

Keywords: COVID-19, SARS-CoV-2, hematopoietic stem cells, natural killer cells

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INTRODUCTION

This year, our attention is focused a lot on the outbreak of Coronavirus Disease (hereafter, COVID-19), which was officially designated as a global pandemic in April 2020 by WHO.[1,2] The viral spread is increasing dramatically within a short period.[3] According to data collection from 213 countries who are currently battling COVID-19, the total positive number of COVID-19 stood at 1,524,161 cases, and the number of deaths reached 92,941 cases.[1] The prevalence of COVID-19 cases in Indonesia as of June 9, 2020, furthermore, has been confirmed 32,076 positive cases, with 1,923 death cases.[4]

COVID-19 is an infectious disease caused by the SARS-CoV-2 virus.[5,6] Like a zoonotic virus, SARS-CoV-2 was transmitted from animals to humans.[7] On its viral infection mechanism, the virus infiltrates to the cells and begins to replicate/reproduce itself using a host cell machine, while causing damages to the host cell.[8] The respiratory system, moreover, is the target of the SARS-CoV-2 virus, which further infects the alveoli cells in the lungs.[6]

In patients with mild infection of COVID-19, there was no increase in chemokine and proinflammatory cytokines, similar results were found even in patients who showed symptoms.[5] On the other hand, a higher neutrophil-lymphocyte ratio, lower leukocytes, and lymphocyte count, and a lower percentage of eosinophils, basophils, and monocytes are observed in patients with severe symptoms, as well as a high level of pro-inflammatory cytokines.[5,9] Acute Respiratory Distress Syndrome (ARDS) is the main cause of death in COVID-19 cases caused by an uncontrolled systemic inflammatory response due to pro-inflammatory cytokines and chemokines that are released in large amounts, which also called as the cytokine storm.[5,10,11] Functional disability will be inevitable due to lung deterioration and fibrosis if this enormous immune response continues to happen.[10,12]

Antiviral therapy, thus, is chosen to treat COVID-19 by referring to therapies used during the previous pandemics, namely MERS and SARS pandemics.[8,13,14] The Republic of China National Health Commission (NHC) has included antiviral therapy as an experimental treatment for COVID-19 in the latest version of the Guidelines for the Prevention, Diagnosis, and Treatment of Novel Coronavirus-induced Pneumonia.[15] Antibiotic therapy, moreover, is also used in conjunction with antiviral therapy.[16] Recently, stem cell-based biological, medical therapy is selected as an optional treatment for handling COVID-19 due to its potential viability.[17]

Hematopoietic stem cells (HSCs) as a multipotent progenitor cell that can be derived into various types of blood cells.[18] HSCs are found in peripheral blood mononuclear cells (PBMCs), bone marrow, or umbilical cord blood.[18,19] HSCs isolation and culture derived from PBMCs and bone marrow must express CD73, CD90, and/or CD105 markers, and not express CD14, CD34, CD45 markers.[18] HSCs further change the profile of cytokine secretion from NK cells, dendritic cells, and naive and effector T cells to induce a more progressive phenotype and anti-inflammatory.[19] The secretion of Tumor Necrosis Factor-alpha (TNF-α) and Interferon-gamma (IFN-γ) as pro-inflammatory cytokines are decreased, while Interleukin-4 (IL-4) and Interleukin-10 (IL-10) are more suppressive stimulated.[18] The other factors, nonetheless, may also be involved, including Interleukin-6 (IL-6), Interleukin-10 (IL-10), Transforming Growth Factor-beta1 (TGF-β1), hepatocyte growth factor, nitric oxide, prostaglandin E2, and possible indoleamine 2, 3-dioxygenase.[19] Numerous evidence claims that HSCs are anti-inflammatory, immunosuppressive, and can be transplanted to incompatible individuals, even though the specific mechanism has not been clarified yet.[20]

Another cell, Natural killer (NK), is inborn lymphocytes that serve as the first defense line against tumor cells and viral infections.[21,22] NK cells have numerous mechanisms to kill cells infected by the virus, including cytolytic granules exocytosis and the potency of extracellular death receptors.[23,24] While the main work describes their antitumor activities, NK cells also play an important role in controlling certain infections, especially viral infections.[25] Granulocyte-macrophage colony-stimulating factor (GM-CSF), also known as a multifunctional cytokine, plays a role as generation and cytotoxicity of NK cells and stimulates macrophages to be antitumor and antimicrobial action.[26] IL-2, furthermore, is needed for the activation and proliferation of many different types of cells, such as NK and T lymphocytes.[27] After being activated by IL-2, NK cells get an increased cytotoxic function (namely lymphokine-activated killer cell, also known as LAK cell), knowing a wider range of targets and killing with heightened lytic potential.[24,27]

From the description above, therefore, the authors aim to conduct an in vitro study concerning the potency of hematopoietic stem cells (HSCs) and natural killer (NK) cells in handling COVID-19 (SARS-CoV-2 virus) using Indonesian virus isolates.

MATERIALS AND METHODS

Materials

α-Minimum Essential Media (α-MEM), Fetal Bovine Serum (FBS), Penicillin-Streptomycin, L-Glutamine, $NaHCO_3$, and HEPES Medium from Gibco®-Thermofisher Scientific, USA; Amphotericin B solution and Phosphate Buffer Saline (PBS) from Sigma-Aldrich®, USA; Collagenase type IV from Worthington®, USA; culture dishes, CO_2 incubator, centrifuge, microscope fluorescence, and polymerase chain reaction (qPCR) machine.

Sample Collection

The SARS-CoV-2 isolates were collected from three confirmed patients with informed consent and ethical approval clearance at Universitas Airlangga Hospital – Tropical Infectious Disease Hospital, Surabaya. Viral Transport Medium (VTM) consisting of Gentamycin sulfate (100µg/ml) and Amphotericin B (0,5µg/ml) were used to collect the patient's sputum. Once samples were collected, the virus isolates were then stored at Biosafety Level 3 (BSL-3) Laboratory in the Institute of Tropical Disease, Universitas Airlangga, Surabaya. Samples were transferred into the new falcon tube, followed by vortexed for 5 minutes and centrifuged at 13.000 rpm for 10 minutes. Each sample was labeled as P1, P2, and P3. After that, the supernatant of each sample was aliquoted in cryotubes and preserved in -80°C deep freezer for long-term use.

Hematopoietic Stem Cells (HSCs) Isolation

The HSCs were obtained from peripheral blood mononuclear cells (PBMCs) of a healthy person and cultured at the Stem Cell Research and Development Center, Universitas Airlangga, Surabaya. The cell culture process took two weeks with changing new medium every three days and adding mitogen GM-CSF (20ng/ml) and IL-2 (100IU/ml). After two weeks of cell culture, hematopoietic stem cells were confirmed with a CD34 marker using immunocytochemistry and seeded $5x10^5$ in 12 multi well-plate each well.

Natural Killer (NK) Cells Isolation

The NK cells were accumulated from peripheral blood mononuclear cells (PBMCs) of a healthy person and cultured at the Stem Cell Research and Development Center, Universitas Airlangga, Surabaya. The cell culture process took two weeks with changing new medium every three days and adding mitogen GM-CSF (20ng/ml) and IL-2 (100IU/ml). After two weeks of cell culture, hematopoietic stem cells were confirmed with a CD56 marker using immunocytochemistry and seeded $5x10^5$ in 12 multi well-plate each well.

Co-cultivation of HSC, NK cell and SARS-CoV-2 in Vitro

Co-cultivation of HSCs, NK cells, and SARS- CoV-2 isolates were performed at Biosafety Level 3. Each sample of P1, P2, and P3 measuring as much as 200 µl was incubated for 72 hours in the CO2 incubator. The collected-supernatant was centrifuged at 5000 rpm for 5 minutes; then, the supernatant and the cell pellet was separated. After that, each sample was extracted with GENEAID RNA extraction kit, and followed by proceeding to check the amount of copy DNA with qRT-PCR.

Pro-viral Load determination

SEEGENE COVID-19 detection kit was used to determine the pro-viral load, which detected three target genes, which are N gene, E gene, and RdRP gene. ABI Prism 7500 Sequence Detector System from Applied Biosystems®, USA, moreover, was used to perform amplification and data acquisition.

RESULTS

The confirmations of HSCs with the CD34 marker and NK cells with the CD56 marker are shown in Figure 1 (a,b), emitting green fluorescence.

A group of Figure 2 displayed the co-cultivation of HSCs and 50 µl, 100 µl, 150 µl, and 200 µl of each sample after 72 hours when the viral load was then determined with qRT-PCR.

A group of Figure 3 presents the co-cultivation of NK cells and 200 µl of each sample P1, P2, and P3 after 72 hours the viral load was then determined with qRT-PCR.

Once the virus planted on HSCs and NK cells, cells and supernatants were taken from existing cell plates, and the viral load was calculated from all plates using Polymerase Chain Reaction (PCR) machine, as shown in Table 1 and Figure 4.

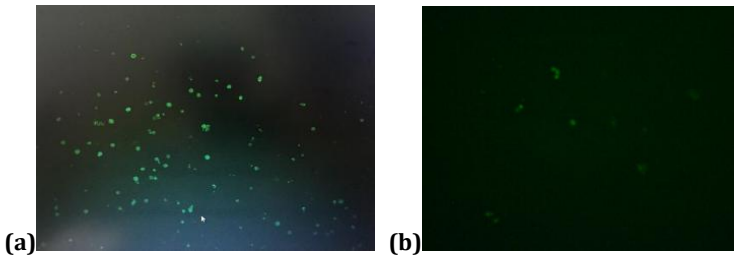

Figure 1. (a) HSCs confirmed with the CD34 marker, **(b)** NK cells confirmed with the CD56 marker

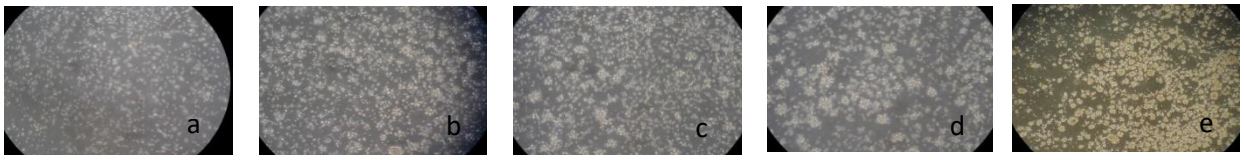

Figure 2. Microscopic Images of HSCs after Planting Viruses after 72 hours **(a)** Control HSCs, **(b)** HSC with 50µl viruses, **(c)** HSCs with 100µl viruses, **(d)** HSCs with 150µl viruses, **(e)** HSCs with 200µl viruses

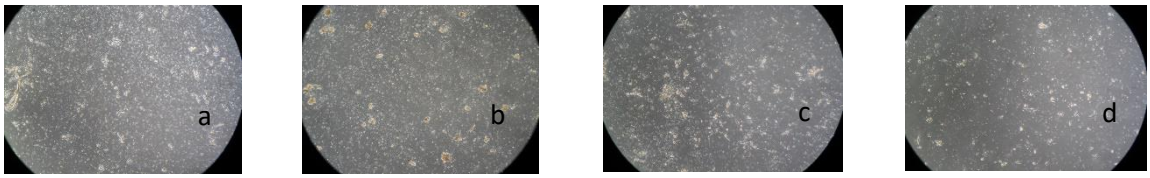

Figure 3. (a) Control cells of NK, **(b)** 72 hours after co-cultivation of P1 sample, **(c)** 72 hours after co-cultivation of P2 sample, **(d)** 72 hours after co-cultivation of P3 sample

Table 1. Data of the viral load on cells and supernatants after augmentation of HSCs

No.	Virus Dosage		MoI	Period	Viral Load
1.	100µl		0.00416	0 hours	20.8 Copy/µl
				24 hours	0 Copy/µl
				48 hours	0 Copy/µl
				72 hours	0 Copy/µl
1.	150µl		0.00624	0 hours	20.8 Copy/µl
				24 hours	0 Copy/µl
				48 hours	0 Copy/µl
				72 hours	0 Copy/µl
2.	200µl		0.00832	0 hours	20.8 Copy/µl
				24 hours	0 Copy/µl
				48 hours	0 Copy/µl
				72 hours	0 Copy/µl

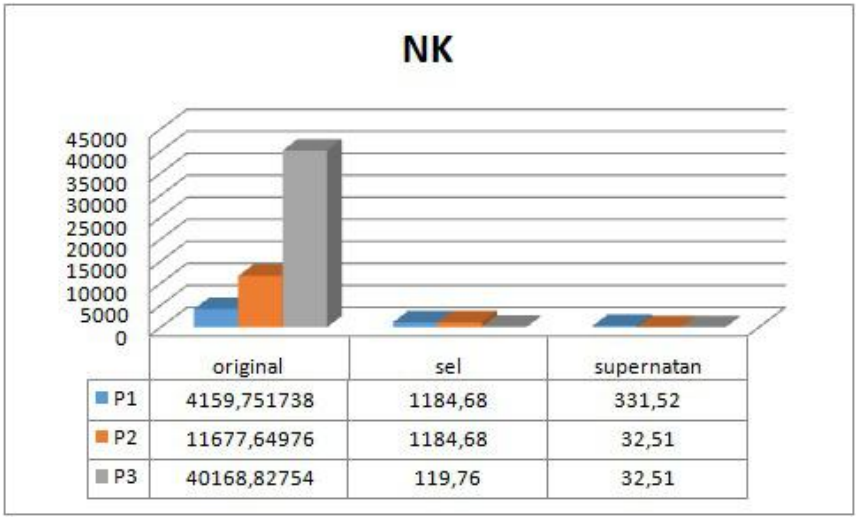

Figure 4. Viral Load of samples after 72 hours co-cultivation NK cells and Indonesian SARS- CoV-2 isolates

DISCUSSION

The immune response against viral infections depends on the effector mechanisms from the innate and adaptive immune responses.[28] The cytotoxicity mediated by cytotoxic CD8+ T cells and NK cells is responsible for executing infected cells.[22] Moreover, NK cells are the potential to mediate antibody-dependent cellular cytotoxicity (ADCC) over the receptor CD16 by binding to antibodies opsonizing infected cells that lead to apoptosis. NCAM-1 (CD56) is expressed by classical NK cells on its membranes in high or low intensity or not express CD16 and lacks express CD3.[25]

Reverse transcription loop-mediated isothermal amplification (RT-LAMP), reverse-transcription polymerase chain reaction (RT-PCR), and real-time RT-PCR (rRT-PCR) are the current gold standards of diagnostic tests to identify the presence of coronavirus.[1,13] Two one-step quantitative RT-PCR (qRT-PCR) is developed to detect two different regions (ORF1b and N) of the SARS-CoV-2 genome to identify patients earlier. Three novels RT-PCR are developed to target the nucleocapsid (N) genes, spike (S), and RNA-dependent RNA polymerase (RdRp)/helicase (Hel). From these three novel assays, the COVID-19-RdRp/Hel has the lowest limit of detection in vitro, and strongly sensitive and specific assays may improve the laboratory diagnosis of COVID-19. The RdRp gene assay, together with the one-step RT-PCR system, is not more sensitive than the SARS-CoV E gene assay.[13]

HSCs as multipotent progenitor cells can rearrange, renew, and differentiate into many various types of blood cells, so it is probably the best type of stem cell with a large-scale of application for the treatment of numerous diseases.[18,19] HSCs change the profile of cytokine secretion from NK cells, dendritic cells, naive and effector T cells to induce a more progressive phenotype and anti-inflammatory, decrease the secretion of TNF-α and IFN-γ as pro-inflammatory cytokines, whereas IL-4 and IL-10 more suppressive stimulated.[19] Other factors may also be involved, such as IL-6, IL-10, TGF-β1, hepatocyte growth factor, nitric oxide, prostaglandin E2, and possible indoleamine 2, 3-dioxygenase. The evidence further claims that HSCs are anti-inflammatory and immunoregulatory, even though the specific mechanism has not been clarified yet.[20]

An increase in IL-10 levels and decreases in TNF-α levels and IL-2 levels in all-time variations and multiplicity of infection (MoI) on the cytokines examination are further discovered. As presented in Table 1, the value of the viral load was decreased. The amount of viral load at the initial administration of HSCs was 20.8 copies/µl in all variants of MoI. After the administration of HSCs, the viral load became 0 copies µl in all-time variants: 24 hours, 48 hours, and 72 hours. The findings further indicate that HSCs can eliminate viruses in cells and supernatants after 24 hours of inoculation. The virus binding to the plasma membrane of the cells is a crucial step in viral infection. The susceptibility of cells to infections depends on the different surface receptor expression. Specifically, the integrin adhesion receptor plays a vital role in mediating the binding and internalization of some viruses. Different integrin receptors expression on early HSC even were noted. Hence, it can be conceived that these receptors mediate the interaction between HSCs and some viruses.[19]

The increase in anti-inflammatory cytokines and the strong decrease in inflammatory cytokines, thereby the virus becomes inactive, are the other mechanisms of HSCs in terminating viruses.[20] HSCs differentiate into the immune cell lineage that leads to virus phagocytosis, and the damaged virus is discharged from the cell.[19,20] From the results, therefore, it is suggested for HSCs to be given to COVID-19 patients in combination with other therapeutic regimens.

As Figure 3 displayed, after 72 hours of co-cultivation of both supernatant and the cell pellet confirmed with qRT-PCR to determine the viral load, the amount of viral load each sample had decreased from the original amount and each showed a decrease of more than 50%. Besides, there was no reduction in the number of NK cells that had been co-cultivated. On the contrary, there was a significant increase in the number of viruses, although the addition of NK cells has inactivated the virus. The result is probably caused by the functions of NK cells, which are related to NCR (NKp46, 44, and 30) that can bind glycoprotein from the virus so that the virus becomes inactive, besides the release of cytokines that cause the virus to be inactivated.

Natural killer (NK) cells, as the critical effector in immunity, play an essential role in the first line of host defense against viral infection by eliminating the infected cells without early antigen stimulation.[25] NK cells have a complex receptor repertoire in inactivating and inhibitory form.[23] After NK cells stimulation, the activating signaling leads to a downstream cascade of kinase activation with the final exocytosis of cytotoxic granules and resulting in the terminating of target cells.[24] The balance of inhibitory and activating receptors and some costimulatory molecules are controlling the cytotoxicity of NK cells.[25] For NK cells to reach a productive response, a critical threshold of signaling must be achieved.[30] Furthermore, virus-infected respiratory epithelial cells release inflammatory chemokines that recruit NK cells to the site of infection.[13]

NK cells demonstrate cytotoxic effects because of direct or indirect target recognition.[24] In the direct target recognition, the identification happens over a general signal from surface receptors of NK cells that receive activating and inhibiting environmental signals.[30] Surface glycoproteins present on all nucleated cells, including major histocompatibility complex I (MHC I) or viral antigens can be molecules recognized by NK cells.[25] The effect of precise target recognition is the killing mechanisms activation, in NK cells: exocytosis of cytotoxic granules or death receptor-mediated cytotoxicity.[21,22,31] Cytotoxic granules contain pore-forming protein, granzymes-serine proteases, and perforin. Perforin produces pores in the target cell membrane, which allows granzymes to get in the cell and begin the apoptosis.[30]

CONCLUSION

The administration of HSCs in patients with COVID-19 can terminate the SARS-CoV-2 virus. During viral infections, the SARS-CoV-2 virus and NK cells are in a constant state of battle. NK cells react to terminate the invading viruses through the increased activating signals or recognition of the missing self. Nevertheless, many viruses have developed different strategies to modulate NK cell activities. Still, this study has shown that NK cells play a critical role in decreasing the viral load of the SARS-CoV-2 virus. Even though this study is an in vitro, it could be the theoretical basis to develop other alternative stem cell-based therapies in handling COVID-19 in Indonesia, particularly and in the globe generally.

Abbreviations

HSCs : Hematopoietic Stem Cell
NK : Natural Killer
MoI : Multiple of Infection
PCR : Polymerase Chain Reaction
SARS : Severe Acute Respiratory Syndrome
MERS : Middle East Respiratory Syndrome
IFN : Interferon
TNF : Tumor Necrosis Factor
IL : Interleukin
ARDS : Acute Respiratory Distress Syndrome
TGF : Transforming Growth Factor
CD : Cluster of Differentiation
PBMCs : Peripheal Blood Mononuclear Cells
BSL-3 : Bio Safety Level 3
RNA : Ribonucleic acid
DNA : Deoxyribonucleic acid
CPE : Cytopathic Effect
Alpha MEM : Alpha Minimum Essential Medium
GM-CSF : Granulocyte-Macrophage Colony Stimulating Factor
HEPES : (N-2-hydroxyethyl piperazine-N-2-ethane sulfonic acid) Medium
$NaHCO_3$: Natrium bicarbonate

Declarations

Ethics approval

The patient's sputum sampling requires an ethical test that was submitted to the Universitas Airlangga Hospital Ethics Commission and has been approved, as evidenced by the issuance of the ethics-worthy certificate number 136/KEP/2020 on April 20, 2020, following the regulatory guidelines of the country.

Consent to publication

Not applicable.

Availability of data and material

Availability of data and materials.

Competing interests

The authors report no conflicts of interest in this work.

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The authors received no specific funding for this work.

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REFERENCES

1. Hu Y, Sun J, Dai Z, Deng H, Li X, Huang Q, et al. Prevalence and severity of corona virus disease 2019 (COVID-19): A systematic review and meta-analysis. J Clin Virol. Elsevier; 2020;104371. https://doi.org/10.1016/j.jcv.2020.104371
2. WHO Indonesia. Coronavirus Disease 2019 (COVID-19) Situation Report - 4. Jakarta; 2020.
3. Harapan H, Itoh N, Yufika A, Winardi W, Keam S, Te H, et al. Coronavirus disease 2019 (COVID-19): A literature review. J Infect Public Health. Elsevier; 2020; https://doi.org/10.1016/j.jiph.2020.03.019
4. Ministry of Health of the Republic of Indonesia. COVID-19 Update until July 28 2020. Jakarta; 2020.
5. Nile SH, Nile A, Qiu J, Li L, Jia X, Kai G. COVID-19:

Pathogenesis, cytokine storm and therapeutic potential of interferons. Cytokine Growth Factor Rev. 2020;53:66–70.
6. Chhikara BS, Rathi B, Singh J, Poonam FNU. Corona virus SARS-CoV-2 disease COVID-19: Infection, prevention and clinical advances of the prospective chemical drug therapeutics. Chem Biol Lett. 2020;7:63–72.
7. Hu B, Ge X, Wang L-F, Shi Z. Bat origin of human coronaviruses. Virol J. 2015;12:221.
8. 8. Wan Y, Shang J, Sun S, Tai W, Chen J, Geng Q, et al. Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry. J Virol. 2019;94:1–15.
9. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. Nat Rev Immunol. Springer US; 2020;20:355–62.
10. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395:507–13. https://doi.org/10.1016/S0140-6736(20)30211-7
11. Lai C-C, Shih T-P, Ko W-C, Tang H-J, Hsueh P-R. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and corona virus disease-2019 (COVID-19): the epidemic and the challenges. Int J Antimicrob Agents. Elsevier; 2020;105924. https://doi.org/10.1016/j.ijantimicag.2020.105924
12. Susilo A, Rumende CM, Pitoyo CW, Santoso WD, Yulianti M, Herikurniawan H, et al. Coronavirus Disease 2019: Tinjauan Literatur Terkini. J Penyakit Dalam Indones. 2020;7:45–67. http://dx.doi.org/10.7454/jpdi.v7i1.415
13. Zhai P, Ding Y, Wu X, Long J, Zhong Y, Li Y. The epidemiology, diagnosis and treatment of COVID-19. Int J Antimicrob Agents. Elsevier; 2020;105955.
14. De Wit E, Van Doremalen N, Falzarano D, Munster VJ. SARS and MERS: Recent insights into emerging coronaviruses. Nat Rev Microbiol. Nature Publishing Group; 2016;14:523–34.
15. Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Discov Ther. International Research and Cooperation Association for Bio & Socio-Sciences; 2020;14:58–60. https://doi.org/10.5582/ddt.2020.01012
16. Jin Y-H, Cai L, Cheng Z-S, Cheng H, Deng T, Fan Y-P, et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). Mil Med Res. Springer;2020;7:4. https://doi.org/10.1186/s40779-020-00246-8
17. Chen J, Hu C, Chen L, Tang L, Zhu Y, Xu X, et al. Clinical study of mesenchymal stem cell treating acute respiratory distress syndrome induced by epidemic Influenza A (H7N9) infection, a hint for COVID-19 treatment. Engineering. Elsevier; 2020; https://doi.org/10.1016/j.eng.2020.02.006
18. Domen J, Wagers A, Weissman IL. Bone marrow (hematopoietic) stem cells. Regen Med. Stem Cell Information from the National Institutes of Health Resource; 2006;13.
19. Kolb-Mäurer A, Goebel W. Susceptibility of hematopoietic stem cells to pathogens: role in virus/bacteria tropism and pathogenesis. FEMS Microbiol Lett. Blackwell Publishing Ltd Oxford, UK; 2003;226:203–7. https://doi.org/10.1016/S0378-1097(03)00643-8
20. Sumorejo P, Purnama DS, Miatmoko A, Nasronudin N, Dinaryanti A, Karsari D, et al. The Potential of Hematopoietic Stem Cells (Hsc) Against Sars-Cov-2 (Covid-19) With Virus Isolates From Indonesia (In Vitro Study). 2020; https://orcid.org/0000-0002-6144-2481
21. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol. Nature Publishing Group; 2008;9:503–10.
22. Fatmariyanti S, Lutfi D. Effectiveness of Natural Killer (NK) Cells in peripheral Blood stem-Cell Towards Expression Of EZH\Ki-67 and Apoptosis in Retinoblastoma (RB) Cells Culturu. Med Sci Orig Investigation. Medicine Science; 2015;50:1–3.
23. Lodoen MB, Lanier LL. Natural killer cells as an initial defense against pathogens. Curr Opin Immunol. Elsevier; 2006;18:391–8. https://doi.org/10.1016/j.coi.2006.05.002
24. Smyth MJ, Cretney E, Kelly JM, Westwood JA, Street SEA, Yagita H, et al. Activation of NK cell cytotoxicity. Mol Immunol. Elsevier; 2005;42:501–10. https://doi.org/10.1016/j.molimm.2004.07.034
25. Amorim CF, Carvalho NB, Neto JA, Santos SB, Grassi MFR, Carvalho LP, et al. The role of NK cells in the control of viral infection in HTLV-1 carriers. J Immunol Res. Hindawi; 2019;2019. https://doi.org/10.1155/2019/6574828
26. Tarr PE. Granulocyte-macrophage colony-stimulating factor and the immune system. Med Oncol. Springer; 1996;13:133–40.
27. Yu T-K, Caudell EG, Smid C, Grimm EA. IL-2 activation of NK cells: involvement of MKK1/2/ERK but not p38 kinase pathway. J Immunol. Am Assoc Immnol;2000;164:6244–51. https://doi.org/10.4049/jimmunol.164.12.6244
28. Pulendran B, Oh JZ, Nakaya HI, Ravindran R, Kazmin DA. Immunity to viruses: Learning from successful human vaccines. Immunol Rev. 2013;255:243–55. https://doi.org/10.1111/imr.12099
29. Lozito TP, Kolf CM, Tuan RS. Microenvironmental regulation of adult mesenchymal stem cells. Regul networks stem cells. Springer; 2009. p. 185–210.
30. Grudzien M, Rapak A. Effect of natural compounds on NK cell activation. J Immunol Res. Hindawi; 2018;2018. https://doi.org/10.1155/2018/4868417
31. Chapter T. Chapter 13 – Immunity to Infection. Prim. to Immune Response. 2014.