Thesis progression

Thesis title: Discriminating Individuals with High and Low Immunosenescent Profiles by Using Blood Serum and Their Components Detected by Raman Spectroscopy

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1. Introduction

Immunosenescence is an age-related change in immune system. This condition lead to the decline in immune system and characterized by decreased immune response resulting in susceptibility to infections, decreased vaccination response, increased expression of pro-inflammatory cytokines which contributes to inflammation-related diseased, increased autoimmune events, and an increased risk of age-related diseases (Aiello et al., 2019; Lian et al., 2020). These age-related changes occur in innate and adaptive immune cells, altering their numbers and functions. One of the important immune cells is CD4+ T cells, due to its crucial function to stimulate other immune cells such as macrophages, B cells, and CD8+ T cells. The disruptions in the T cell pool and persistent inflammation could lead to premature aging of other immune cells. Furthermore, WHO recognize ageing at the biological level can lead to gradual decrease in physical and mental capacity, increasing risk of disease and death (Ageing and Health, n.d.). Early detection of immunosenescence in individual is important for disease risk management. It could help clinicians to adjust the specific healthcare on the patient based on their immune status.

Immunosenescent T cells are identified by flow cytometry using the related markers and throughout literature often called as senescent T cells (Slaets et al., 2024). Flow cytometry is a powerful technique and has been utilized to assess the circulating immunophenotype in COVID-19 (Bencivenga et al., 2020), vasculitis (H. Zhang et al., 2019), or

healthy individual (Sun et al., 2022). However, some challenges arise in using flow cytometry, such as the need of expertise in sample preparation and instrument operation, instrument accessibility, and subjective data interpretation. Therefore, other sample source and methods that is easy to perform, rapid and cost-effective for detection of immunosenescence need to be explored.

Raman spectroscopy is an instrument that measure the raman scattering phenomenon. It has been risen as a diagnostic tool in recent years. This is due to raman spectroscopy advantages such as the spectra follows the sample composition, minimum sample preparation, and versatility due to various sample type can be measured. Raman spectroscopy technique has been used to study cellular senescence and aging in fibroblasts (Eberhardt et al., 2017), cancer cells (Ghislanzoni et al., 2023), oocytes (Bogliolo et al., 2013), mesenchymal stem cells (MSCs) (Bai et al., 2015), and red blood cells (RBCs) (Lenzi et al., 2021). Surface-enhanced Raman spectroscopy (SERS) is powerful analytical technique for molecule detection that can investigate conformational changes in complex biological molecules. SERS utilize enhanced Raman scattering, allowing for the detection of molecules even at very low concentrations, and more sensitive compared to standard Raman Spectroscopy.

Therefore, this study aims to differentiate individuals with high and low percentage of pathogenic/immunosenescent CD4+ T cells are possible to be differentiated by analyzing its serum and exosome by using Raman spectroscopy/SERS

2. Hypothesis and Objective

2.1 Hypothesis

Individuals with high and low percentage of pathogenic/immunosenescent CD4+ T cells are possible to be differentiated by analyzing its serum and exosome by using Raman spectroscopy/SERS

2.2 Objective

To differentiate the immune profiling from serum and small biomolecule (extracellular vesicle, exosome) of individuals with high and low percentage of pathogenic / immunosenescent CD4+ T cells by using portable Raman spectroscopy and SERS

3. Materials and methods

3.1 Samples

Whole blood will be collected from healthy individuals according to the criteria (18-60 years old). Whole blood will be collected in heparin/EDTA tubes and tubes without anticoagulant. Heparinized/EDTA whole blood will be used for surface staining. Meanwhile the whole blood without anticoagulant will be let to clot, then subjected for serum isolation by centrifugation at 1,000–2,000 x g for 10 minutes in a cold condition. The serum will be collected in sterile vessels and stored at -80°C until further use.

3.2 Exosome Isolation

Exosome isolation will be done by using ExoQuick™ Exosome Precipitation Solution (System Biosciences, Palo Alto, CA, USA, cat no. EXOQ5A-1) or Total Exosome Isolation Reagent (from serum) (Invitrogen, Life Technology, Carlsbad, CA, USA, cat no. 4478360). Protocol will be done according to the manufacturer's instructions. The isolated exosome will be stored at 80 C prior to use.

3.3 Measurement of Cell Surface Markers of CD4+ T Cells (Flow cytometry)

Fresh peripheral whole blood (100 µL) in heparin/EDTA anticoagulant will be stained for 15 minutes in the dark with multicolor fluorochromes conjugated with monoclonal antibodies (mAbs) as follows: CD3-fluorescein Isothiocyanate (FITC, BD Pharmingen, USA), CD4-Allophycocyanin-Cyanine 7 (APC-Cy7, xxxx), CD28-phycoerythrin-cyanine 7 (PE-Cy7, BioLegend, USA), CD314-Allophycocyanin (APC, xxx), CD57-phycoerythrin (PE, BioLegend, USA), KLRG1 (MAFA)- Brilliant Violet 785 (BV785, BioLegend, USA). Fluorescence Minus Three (FMT) of APC, PE, and BC785 will used for observing nonspecific binding and setting cutoffs for CD314, CD57, and KLRG1 expression. Then, red blood cells will be lysed by using BD FACS lysing solution (BD Biosciences, USA) for 10 min in the dark before 1 wash with 1x

phosphate buffer saline (PBS). The prepared samples will be measured and analyzed using BD FACSLyric flow cytometer (BD Biosciences, USA).

3.4 Measurement of intracellular cytokine

Peripheral blood mononuclear cells (PBMCs) from a total of x heparin/EDTA blood samples will be isolated using xxxxx. Cells were stimulated with xxxxxxx. Afterward, cells were fixed with 2% formaldehyde and treated with a permeabilization/wash buffer before the intracellular staining of interleukin (IL)-17 (PerCP-Cy5) and interferon-gamma (IFN-γ) (BV421). The samples were then measured with a BD FACSLyric™ flow cytometer. Unstimulated sample (medium) will be measured in parallel.

3.2.5 Raman and SERS Spectral acquisition

Sample spectra will be recorded using portable Raman Spectrometer. For Raman measurement, glass wrapped with aluminum foil will be used for platform. Meanwhile, for SERS, xxx substrate will be used for platform. Three spectra will be collected for each sample.

4. Results

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5. Conclusion

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6. References

Ageing and health. (n.d.). Retrieved December 28, 2025, from https://www.who.int/news-room/fact-sheets/detail/ageing-and-health

Aiello, A., Farzaneh, F., Candore, G., Caruso, C., Davinelli, S., Gambino, C. M., Ligotti, M. E., Zareian, N., & Accardi, G. (2019). Immunosenescence and Its Hallmarks: How to Oppose Aging Strategically? A Review of Potential Options for Therapeutic Intervention. Frontiers in Immunology, 10. https://doi.org/10.3389/fimmu.2019.02247

Bai, H., Li, H., Han, Z., Zhang, C., Zhao, J., Miao, C., Yan, S., Mao, A., Zhao, H., & Han, Z. (2015). Label-free assessment of replicative senescence in mesenchymal stem cells by

Raman microspectroscopy. Biomedical Optics Express, 6(11), 4493–4500. https://doi.org/10.1364/BOE.6.004493

Bencivenga, L., Rengo, G., & Varricchi, G. (2020). Elderly at time of COronaVIrus disease 2019 (COVID-19): Possible role of immunosenescence and malnutrition. GeroScience, 42(4), 1089–1092. https://doi.org/10.1007/s11357-020-00218-9

Bogliolo, L., Murrone, O., Di Emidio, G., Piccinini, M., Ariu, F., Ledda, S., & Tatone, C. (2013). Raman spectroscopy-based approach to detect aging-related oxidative damage in the mouse oocyte. Journal of Assisted Reproduction and Genetics, 30(7), 877–882. https://doi.org/10.1007/s10815-013-0046-6

Ghislanzoni, S., Kang, J. W., Bresci, A., Masella, A., Kobayashi-Kirschvink, K. J., Polli, D., Bongarzone, I., & So, P. T. C. (2023). Optical Diffraction Tomography and Raman Confocal Microscopy for the Investigation of Vacuoles Associated with Cancer Senescent Engulfing Cells. Biosensors, 13(11), 973. https://doi.org/10.3390/bios13110973

Lenzi, E., Dinarelli, S., Longo, G., Girasole, M., & Mussi, V. (2021). Multivariate analysis of mean Raman spectra of erythrocytes for a fast analysis of the biochemical signature of ageing. Talanta, 221, 121442. https://doi.org/10.1016/j.talanta.2020.121442

Lian, J., Yue, Y., Yu, W., & Zhang, Y. (2020). Immunosenescence: A key player in cancer development. Journal of Hematology & Oncology, 13, 151. https://doi.org/10.1186/s13045-020-00986-z

Slaets, H., Veeningen, N., de Keizer, P. L. J., Hellings, N., & Hendrix, S. (2024). Are immunosenescent T cells really senescent? Aging Cell, 23(10), e14300. https://doi.org/10.1111/acel.14300

Sun, H., Kang, X., Chen, X., Cai, L., Li, Y., Yu, J., Wu, C., & Deng, X. (2022). Immunosenescence evaluation of peripheral blood lymphocyte subsets in 957 healthy adults from 20 to 95 years old. Experimental Gerontology, 157, 111615. https://doi.org/10.1016/j.exger.2021.111615

Zhang, H., Watanabe, R., Berry, G. J., Nadler, S. G., Goronzy, J. J., & Weyand, C. M. (2019). CD28 Signaling Controls Metabolic Fitness of Pathogenic T Cells in Medium and Large Vessel Vasculitis. Journal of the American College of Cardiology, 73(14), 1811–1823. https://doi.org/10.1016/j.jacc.2019.01.049