



### **Title of Research:**

21-2-01 Development of evaluation method of inflammatory particles based on alveolar macrophage function.

### **Principal Investigator:**

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### **Summary of Research:**

The number of patients with allergic diseases have increased in developed countries, and it is suggested that particulate pollution such as PM<sub>2.5</sub> might be one of factors of exacerbation of allergic inflammation. In this study, we were focused on alveolar macrophages that engulf and excrete inhaled particles, and we aim to develop methods of evaluation of inflammatory particles focusing on alveolar macrophage functions in response to particles.

Recently, animal-free toxicity testing is recommended in these days, so we tried to develop in vitro evaluation methods using alveolar macrophage cell lines, and cell line ALV3 was we successfully obtained. Then single cell cloning was performed and a stable cell line, ALV3.7, was generated. Next, we examined the expression of surface marker, the responses to inflammatory particles, and comprehensive analysis of gene expression, and found that enhanced gene expression regarding DNA replication and cell cycle were observed. However, ALV3.7 was thought to be maintained the characteristics of alveolar macrophages. In vitro analysis revealed that inflammatory particles stimulated ALV3.7 to induce cell death and subsequent release of intracellular IL-1 $\alpha$ , confirmed by live cell imaging analysis. Furthermore, these particles induced allergic inflammation in mice after the airway sensitization, suggesting that in vitro responses of ALV3.7 reflect to the biological responses in vivo, especially allergic responses.

In conclusion, we have established the alveolar macrophage cell line, ALV3.7 as a useful tool for in vitro assessment of inflammatory particles. In addition, ALV3.7 can be expected to be applied to various chemical evaluation methods without using animals.

### **Timeline:**

March 1, 2023—February 29, 2024

### **Topics:**

Research meeting of LRI by JCIA, oral, "Development of evaluation method of inflammatory particles based on alveolar macrophage function", on-site/online, August 25, 2023.

### **Publications:**

Adachi, T., Inoue, H., Izumi, H. and Kuroda E. "Establishment of a functional alveolar macrophages cell line" The 30<sup>th</sup> Annual Meeting of the Japanese Society of Immunotoxicology, Kanagawa, 2023.9.11.

Kuroda Etsushi "The effect of chemical properties of particulate matter on alveolar macrophage activation" Consortium of Metal Biosciences 2023 Symposium, Gifu, 2023.10.