Title of Research: ***24-6-01***

Development of a coculture system to assess respiratory sensitization using IL-4 production from human Th2 cells as a marker

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Summary of Research:

The development of alternative methods for animal testing of respiratory sensitization has been the subject of an OECD Detailed Review Paper since the year before last, and the final version was recently completed. Our dendritic cell (DC) coculture system (DCsens) and the two-step DC/T coculture system (DC/Tsens), which adds T cells to DCsens, are also included. DCsens is a coculture system using human bronchial epithelial cell line BEAS-2B and human monocyte cell line CD14-ML cells with a scaffold. DC/Tsens is a coculture system where T cells are added to the DC layer stimulated in the DCsens. DC/Tsens uses IL-4 production, a powerful inducer of Th2 differentiation from T cells and an effector molecule of Th2 cells, as its indicator.

Based on studies conducted up to last year, using an allo-specific Th2 cell line enabled the measurement of IL-4 production via ELISA. For a total of 13 chemicals, including 6 skin sensitizing chemicals and 7 respiratory sensitizing chemicals, Evaluation method 1 yielded predictive sensitivity, specificity, and accuracy of approximately 85% each. However, as the number of chemicals increased, sensitivity—the ability to identify respiratory sensitization—began to decline. This evaluation method 1 calculated the cytokine production ratios for IL-2, IFN-γ, and IL-4 using the maximum concentration at which each cytokine showed peak production, even if this maximum concentration differed across the tested concentrations. Therefore, as evaluation method 2, we first calculated the ratio of cytokine production values between concentrations of the same chemical substance and used the maximum value. In this case, since abnormally large values could occur depending on the concentration, we decided to raise the cell survival rate threshold from 20% to 40%. As a result, we evaluated only those chemicals meeting this cell survival rate criterion. This increased sensitivity to 100% for a total of 15 chemicals, including 8 skin sensitizing chemicals and 7 respiratory sensitizing chemicals. We plan to collect data using this survival rate criterion going forward.

Timeline:

March 2025 – February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.

Publications:

1. Yoshimoto T, Katahira Y, Hasegawa H, Qu N, Yamaguchi N, Horio E, Ashikaga S, Mizoguchi I. Prediction of the Respiratory Sensitizing Potential of Chemicals Using DC Coculture and DC/T Cell Coculture Systems. SOT 2025（2025.3.20-24）Orlando, FL, USA
2. Yoshimoto T. Predictive potential of chemical respiratory sensitization using DC coculture (DCsens) and two-step DC/T cell coculture (DC/Tsens) systems. RIFM Respiratory Research Workshop: Future Forward（2025.7.15-16）Mahwah, NJ, USA
3. Mizoguchi I. Development of a 3D coculture system enabling accurate prediction and evaluation of respiratory sensitizing chemicals. The 32nd Annual Meeting of The Japanese Society of Immunotoxicology（2025.9.4-5）Gifu, Japan