Title of Research: **19_R03-01-3**

Development of a novel alternative method for evaluation of sensitizing potential and allergenicity by measuring human T cell activation and differentiation (Key event 4)

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Summary of Research:

Although several *in vitro* assays that predict the sensitizing potential of chemicals have been developed, none can distinguish between chemical respiratory and skin sensitizers. Previously, we established a new three-dimensional dendritic cell (DC) coculture system consisting of a human airway epithelial cell line, immature DCs derived from human peripheral monocytes, and a human lung fibroblast cell line. In this coculture system, compared to typical skin sensitizers, typical respiratory sensitizers showed enhanced mRNA expression in DCs of the key costimulatory molecule OX40 ligand (OX40L), which is important for T helper 2 (Th2) cell differentiation. Herein, we established a new two-step DC/T cell coculture system by adding peripheral allogeneic naive $CD4^+$ T cells to the DCs stimulated in the DC coculture system. In this DC/T cell coculture system, typical respiratory sensitizers but not skin sensitizers enhanced mRNA expression of the predominant Th2 marker IL-4 and its transcription factor GATA-3. To improve the versatility, in place of peripheral monocytes, monocyte-derived proliferating cells called CD14-ML were also used in the DC coculture system. Similar to peripheral monocytes, enhanced mRNA expression of OX40L was observed by typical respiratory sensitizers compared to skin sensitizers. In the 9th term, these cell lines were applied to the DC/T cell coculture system with peripheral allogeneic naive $CD4^+$ T cells, and it was revealed that typical respiratory sensitizers but not skin sensitizers enhance the mRNA expression of IL-4. When allogeneic Th2 cell line was also applied to it, typical respiratory sensitizer enhanced IL-4 mRNA expression, as well as IL-4 secretion. Thus, this DC/T cell coculture system might be useful for discriminating between respiratory and skin sensitizers by differential upregulation of IL-4 in T cells.

Timeline: March 1, 2019-Feberary 28, 2022

Topics:

2021 Annual Meeting of The Japan Chemical Industry Association LRI, oral presentation “Development of a novel alternative method for evaluation of sensitizing potential and allergenicity by measuring human T cell activation and differentiation (Key event 4)” (Web meeting, Aug. 20, 2021)

Publications:

1. Mizoguchi I, et al. A novel coculture system for assessing respiratory sensitizing potential by IL-4 in T cells. ALTEX in press.
2. Yoshimoto et al. Development of a novel alternative method for evaluation of sensitizing potential and allergenicity by measuring human T cell activation. The 34th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments, Web Oral Presentation (Nov. 11-13, 2021, Okinawa)
3. Mizoguchi et al. Development of a novel alternative method for evaluation of sensitizing potential and allergenicity by measuring human T cell activation and differentiation. The 34th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments, Web Oral Presentation (Nov. 11-13, 2021, Okinawa) President’s Special Award