Title of Research:

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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:

Several in vitro assays to predict the sensitizing potential of chemicals have been developed so far. However, these animal testing alternatives cannot distinguish chemical respiratory and skin sensitizers, although the risk management systems for them are quite different. Therefore, we have been aiming at developing a novel in vitro assay, which can discriminate them by taking advantage of the fundamental differences between their modes of function; development of helper T (Th) 2 immune responses, which are critically important for respiratory sensitization. Recently, we established a new 3-dimentional (3D) dendritic cell (DC) coculture system consisting of human airway epithelial cell line, immature DCs derived from human peripheral monocytes, and lung fibroblast cell line. This coculture system was shown to successfully discriminate respiratory sensitizers from skin sensitizers using 6 representative chemical sensitizers by more enhanced mRNA expression of key costimulatory molecule OX40 ligand (OX40L), which is important for Th2 differentiation, in DCs (Mizoguchi et al. Front Immunol. 2017). In this project, we have been further trying to establish a new 2-step DC/T coculture system by introducing T cells in the DC coculture system, in which the Key event 4, that is T cell, in the adverse outcome pathway of sensitization can be used as a marker. To increase the versatility, we are also trying to generate DC progenitor cell lines and T cell lines to apply for it.

In the 8th term, as a DC progenitor cell line, we eventually generated monocytic cell lines by introducing genes related to cell survival and cell cycle into peripheral $CD14^+$ monocytes. When these cell lines were applied for the 3D DC coculture system, the similar more enhanced mRNA expression of OX40L was observed by respiratory sensitizers than skin sensitizers. When these cell lines were then applied for the 3D DC/T coculture system using primary $CD4^+$T cells, the similar more enhanced mRNA expression of IL-4 was also observed by respiratory sensitizers. As the possible molecular mechanism to enhance IL-4 mRNA expression, augmented expression of the transcription factor GATA-3, that is important for Th2 differentiation, was also observed by respiratory sensitizers. Furthermore, we have generated allogenic Th1 and Th2 cells and syngeneic cedar pollen antigen (Cryj1)-specific Th2 cells, and are currently cloning them.

Timeline:

March 1, 2020 - February 28, 2021

Topics:

Oral presentation at JCIA LRI Annual Workshop 2020 “Development of a novel alternative method for evaluation of sensitizing potential and allergenicity by measuring human T cell activation and differentiation (Key event 4)” (On-line, August 21st, 2020)

Publications:

Yoshimoto T. Expectations for immune on-chip. Symposium on New Technology for Cell-based Drug Assay (Web meeting, Jan. 26, 2021)