Research on the effects of chemical substances on children, elderly people, and those with gene disorders

Title of Research:

19_R03-01

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:

Recently, several in vitro assays to predict the sensitizing potential of chemicals have been developed. 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 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 expression of key costimulatory molecule OX40 ligand (OX40L), which is important for Th2 differentiation, in DCs. In the present study, we are currently 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 iPS-derived DC progenitors and T cell lines to apply for it.

In the 7th term, DCs stimulated by 6 representative sensitizers in the DC coculture system were further reacted with allogeneic naive $CD4^+$ T cells in the 2-step DC/T coculture system. As a result, selective up-regulation of IL-4, Th2 differentiation marker, by respiratory sensitizers could be seen after 5-day culture. Furthermore, we generated several iPS-derived DC progenitor lines by transduction of genes related to cell survival and cell cycle. Some of them showed a tendency to selectively up-regulate OX40L by respiratory sensitizer in the 3D DC coculture system as primary monocyte-derived immature DCs did. We are currently still continuing to investigate them.

Timeline:

March 1, 2019 - February 29, 2020

Topics:

Oral presentation “Establishment of a novel in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals” and poster 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)” at JCIA LRI Annual Workshop 2019 (Tokyo, August 30th, 2019)

Publications:

1. Mizoguchi I, Yoshimoto T, et al. Development of a novel 3D co-culture system to evaluate sensitizing potential of chemicals. The 32th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments. (Tsukuba, Nov. 20-22, 2019)
2. Mizoguchi I, Yoshimoto T, et al. Development of a novel alternative method for evaluation of sensitizing potential and allergenicity by measuring human T cell activation and differentiation

in vitro. The 48th Annual Meeting of the Japanese Society for Immunology. (Hamamatsu, Dec. 12-13, 2019)