



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

Title of Research:

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Establishment of in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals

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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 sensitizers and skin sensitizers, although the risk management systems for them are quite different. Therefore, in the present study, we aim 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 is critically important for respiratory sensitization.

So far, we established a new immunocompetent 3D co-culture system consisting of human airway epithelial cells, immature dendritic cells (DCs) derived from human peripheral blood CD14⁺ monocytes, and human fibroblast cells. This co-culture system was demonstrated to predict the respiratory sensitizing potential of chemicals by means of more enhanced expression of key costimulatory molecule OX40Ligand (OX40L), which is important for Th2 differentiation, in DCs. In this term, to further improve the versatility, we utilized iPS cell-derived DCs based on published protocols, although their efficacy is not so good. Therefore, we initially tried to establish a novel method to generate infinitely growing myeloid progenitors by stimulation with various cytokines and/or lentiviral incorporation of genes related to cell cycle and survival. Finally, we have developed several types of immortalized myeloid progenitors. By using these cells-derived DCs, the co-culture system seems to be able to discriminate between skin and respiratory sensitizers as in the case of primary DCs. Moreover, we also tried to develop a novel DC/T co-culture system, which consists of sensitized DCs and allogenic naive CD4⁺ T cells. In this system, we have great advantage that IL-4 up-regulation can be used as a marker for the prediction of respiratory sensitizing potential. Indeed, our preliminary data suggest that selective up-regulation of IL-4 was observed by the stimulation with respiratory sensitizer as compared to that with skin sensitizer.

Timeline:

March 1, 2018 - February 28, 2019

Topics:

2018 Annual Meeting of The Japan Chemical Industry Association LRI, poster presentation "Establishment of a novel in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals" (The Tokyo Shoken Building, Aug. 31, 2018)

Publications:

1. Yoshimoto T, Ohashi M, Hasegawa H, Orii N, Xu M, Owaki T, Mizoguchi I. Development of a novel alternative method by using 3D co-culture system for evaluation of respiratory sensitizing potential. The 45th Annual Meeting of The Japanese Society of Toxicology, Workshop. (Osaka, Jul. 18-10, 2018)
2. Yoshimoto T, Hasegawa H, Orii N, Xu M, Mizoguchi I. Prediction of chemical respiratory



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sensitizers by OX40L expression in dendritic cells using a new 3D co-culture system. The 4th International Conference on Toxicity Testing Alternatives & Translational Toxicology and the 2nd Asian Congress on Alternatives. (Guangzhou, Guangdong, China, Oct. 9-12, 2018)