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

Principal Investigator:

Takayuki Yoshimoto, PhD (Professor, Tokyo Medical University, Institute of Medical Science)
6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan
(tel)+81-3-3351-6141 Ext. 431, (e-mail) yoshimot@tokyo-med.ac.jp

Collaborators:

Izuru Mizoguchi, PhD (Research Associate, Tokyo Medical University, Institute of Medical Science) 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan
(tel) +81-3-3351-6141 Ext. 448, (e-mail) imizo@tokyo-med.ac.jp

Summary of Research:

Recently, the use of animal models in safety testing of chemicals will be significantly limited due to introduction of 3Rs principles of animal experimentations in research wherever possible. Therefore, several in vitro assays to predict the respiratory sensitizing potential of chemicals have been developed. However, these alternative methods 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 respiratory sensitizers from chemical skin sensitizers 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.

First of all, we established an immunocompetent 3D co-culture system of human upper airway using the Alvetex® scaffold from Reinnervate Ltd. It consists of a human airway epithelial cell line BEAS-2B, immature dendritic cells (DCs) derived from peripheral blood $CD14^+$ monocytes, and a fibroblast cell line MRC-5 cultured in individual scaffolds, and these scaffolds were then assembled into a 3D multi-cell tissue model, which more closely mimics the in vivo situation. Then, three typical chemicals for respiratory sensitizers such as phthaldialdehyde, hexamethylene diisocyanate, and trimellitic anhydride and skin sensitizers such as oxaloacetic acid, formaldehyde, and dinitrochlorobenzene disolved in DMSO diluted with medium were added on the 3D co-culture system. After incubation for 9 hours, total RNA was extracted and subjected to real-time RT-PCR to analyze the expression levels of 14 molecules, which are critical for induction of Th2 differentiation and Th2 immune responses such as OX40L, CCR7, IL-10, IL-33, and thymic stromal lymphopoietin. These sensitizers showed similarly augmented expression of DC maturation markers such as CD86, but among these molecules OX40L expression in DCs was most greatly enhanced by the respiratory sensitizers as compared with that by the skin sensitizers.

The present results indicate that this 3D co-culture system has great potential to contribute to the establishment of in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals by means of enhanced expression of key molecules such as OX40L as markers in DCs.

Timeline:

March 1, 2016-Feberary 28, 2017

Topics:

ICCA-LRI and NIHS Workshop, poster presentation “Establishment of a novel in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals” (Awaji Island, June 15-16, 2016)

Publications:

The 45th Annual Meeting of The Japanese Society for Immunology, poster presentation “Development of a novel 3D co-culture system for evaluation of respiratory sensitizing potential” (Okinawa, December 5-7, 2016)