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

Title of Research:

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Development of high sensitivity in vitro assessment system of chemical-mediated hypersensitivity by using serine protease inhibitor-deficient cells
Subtitle: Analysis of in vitro assessment for respiratory allergy

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Summary of Research:

The allergic disorders triggered by various chemicals are separated into two types of diseases, IgE-dependent immediate allergy and IgE-independent chronic allergy. Mast cells, basophils and eosinophils act as the effector cells in both allergic reactions. In particular, basophils and eosinophils rapidly infiltrate into respiratory tissue, and cause airway hyper-responsiveness and airway obstruction. These cells secrete abundant serine proteases as well as chemical mediators and cytokines. Serine proteases, such as elastases, disrupt the basement membrane, leading to the infiltration of inflammatory cells. The serine proteases are repressed by the serine protease inhibitors. Because we found that basophils and eosinophils lacking a secretory leukoprotease inhibitor SLPI produced more cytokines than wild type cells after stimulation with IgE or LPS. Therefore, we have planed to establish the highly sensitive cell line by the deletion of serine protease inhibitors using a human basophilic cell line, KU812-F. Because KU812-F expresses high levels of Serpin b1 and b6, and we have generated stable Serpin b1 knockdown KU812-F cell lines by using lentiviral-delivered sh (short hairpin) RNA. The knockdown KU812-F cells (KD cells) produced IL-6 about 2 times higher than KU812-F after administration with toluene diisocyanate (TDI). KD cells also secreted IL-13 upon stimulation with TDI. In contrast, scramble and wild type KU812-F cells did not produce IL-13. In the present LRI research, we investigated gene alteration between KD cells and scramble KU812-F cells in responses to TDI by mRNA sequencing analysis. We found significant elevation of three genes as follows: CD69 (T cell activation marker), CXCR4 (chemokine receptor), sphingosine-1-phosphate receptor 4 (S1PR4: cell migration). Because commercially available SIPR4 antibodies recognize the intracellular domain, we examined the expression of CD69 and CXCR4 by flow cytometric analysis upon stimulation with TDI and formaldehyde, a skin sensitizer Dinitrochlorobenzene (DNCB), and control Methyl Salicilate. We evaluated the relative fluorescence intensities (RFI) in accordance with human Cell Line Activation Test. KU812-F and KD cells showed higher RFI of CD69 than scramble cells upon TDI and formaldehyde stimulation, whereas all cell lines did not display any alteration of CD69 expression after DNCB and Methyl Salicilate. On the other hand, we could not detect CXCR4 expression in these cell lines. Taken together, our data suggested that analysis of CD69 expression and IL-13 ELISA is a suitable application for the detection of respiratory sensitizes by using Serpin b1 knockdown KU812-F cells.

Timeline:

2016/3/1-2017/2/28

Topics:

Poster presentation entitled “Development of high sensitivity *in vitro* assessment system of chemical-mediated hypersensitivity by using serine protease inhibitor-deficient cells.” at the ICCA-LRI and NIHS Workshop, Awaji Island, Japan, June, 2016