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

Principal Investigator:

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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. In the previous LRI research, we found that a serine protease inhibitor, 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 produced IL-6 about 2 times higher than KU812-F after administration with toluene diisocyanate (TDI). The knockdown 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 mRNA expressions of cytokines that contribute to allergic responses upon stimulated with respiratory sensitizers, TDI and formaldehyde, and contact sensitizers, methyl salicylate and DNCB. KU812-F cells showed high levels of IL-4, 6, 13, and TSLP mRNAs upon TDI stimulation. Although there were no significant differences of mRNA expressions between knockdown and control scramble cells, ELISA assays showed that the knockdown cells, but not scramble cells, produced IL-13, and also demonstrated that the knockdown cells produced more IL-13 than control cells after administration with formaldehyde. The knockdown cells did not secrete IL-6 and 13 when stimulated with contact sensitizers. These results suggested that IL-13 ELISA assay is a suitable application for the detection of respiratory sensitizes using Serpin b1 knockdown KU812-F cells.

Timeline:

2015/3/1-2016/2/28

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 4th annual conference of new LRI, Tokyo, Japan, August 2015