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. In 2011 LRI research, we found that a serine protease inhibitor, secretory leukoprotease inhibitor (SLPI) are expressed in murine basophils and eosinophils. Both *Slpi* $^{-/-}$ (SLPI-deficient) basophils and eosinophils produced more cytokines than wild type (WT) cells after stimulation with IgE or LPS. Therefore, we have planed to establish the highly sensitive cell lines of chemical-mediated hypersensitivity by the deletion of serine protease inhibitors including SLPI. In 2012LRI study, we firstly examined the cytokine responses of murine *Slpi* $^{-/-}$ basophils and eosinophils. As in the case of 2011LRI research, *Slpi* $^{-/-}$ basophils were susceptible for the cell toxicity of the chemicals, demonstrating that murine *Slpi* $^{-/-}$ basophils seemed to be inadequate for the screening of the sensitizers. Conversely, *Slpi* $^{-/-}$ eosinophils showed high viabilities after administration with various chemicals. In addition, IL-6 production stimulated with beryllium sulfate was augmented in *Slpi* $^{-/-}$ eosinophils, suggesting that eosinophils lacking serine protease inhibitors are suitable for the screening of some chemicals. We next investigated the expression profile of the serine protease inhibitors in mast cells, basophils, and eosinophils by DNA microarray analysis. The array studies showed the results as follows. : Mast cells express Serpin b1a and b6a. Basophils express Serpin b1a, b2, and SLPI. Therefore, these serine proteases would be the target genes for the generation of cell lines. We also induced the toluene diisocyanate (TDI)-induced respiratory inflammation. However, since the cellular infiltration was comparable between WT and *Slpi* $^{-/-}$ mice, the methods should be improved. Finally, we tested the cell viability and cytokine responses in the basophilic cell line KU812. KU812 showed high viabilities against various chemicals. KU812 also secreted cytokines in response to several chemicals, suggesting that KU812 lacking serine protease inhibitors would be an ideal risk evaluation model against chemical substances.

Timeline: November 1, 2012 -

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 2th annual conference of new LRI, Tokyo, Japan, August 2012