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 2011 LRI research, we found that a serine protease inhibitor, secretory leukoprotease inhibitor SLPI-deficient (*Slpi* $^{-/-}$) basophils and eosinophils produced more cytokines than wild type 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 2012 LRI research, we found that *Slpi* $^{-/-}$ eosinophils highly produced IL-6 stimulated with beryllium sulfate. The DNA microarray analyzes revealed the expression profile of serine protease inhibitors in mast cells, basophils and eosinophils. Moreover, a human basophilic cell line KU812 secreted cytokines in response to several chemicals. In the present 2013 LRI study, we examined the cytokine responses by a human eosinophilc cell line EoL-1. EoL-1 produced IL-6 after stimulation with Nickel compounds among various chemicals. We next investigated the expression of serine protease inhibitors in KU812 and KU812 substrain, KU812-F. KU812-F expresses higher levels of Serpin b1 and b6 than KU812. In addition, IL-6 production was augmented in KU812-F than KU812 cells. Finally, we 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 produced IL-6 in response to 9 kinds of chemicals that KU812-F showed no responses. The knockdown cells also secreted IL-13 upon stimulation with TDI that KU812-F did not response. These results suggested that Serpin b1 knockdown KU812-F cell would be an ideal risk evaluation tool against chemicals.

Timeline:

2013/1/1-2015/2/28

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 3th annual conference of new LRI, Tokyo, Japan, August 2014