Letter to the Editor

Involvement of taste receptors in the effectiveness of sublingual immunotherapy

Dear Editor,

Japanese cedar pollinosis (JCP) is a specific seasonal allergic disease which affects ~30% of the Japanese population, between February and April, every year. Apart from a series of symptom-reliever medications, allergen-specific immunotherapy (AIT) is one of the most effective treatments for JCP. After several years of relying on the application of subcutaneous immunotherapy (SCIT) with standardized Japanese cedar pollen extract (since the 1960s), the use of sublingual immunotherapy (SLIT) was approved in 2014. In addition to the numerous clinical and scientific evidences pertaining to its effectiveness and safety on JCP including the results of randomized, placebo-controlled, double-blind studies, SLIT is also easier to administer and safer than SCIT, in which a systemic allergen injection is required and severe side effects include fatal anaphylaxis. However, the underlying mechanisms through which SLIT and SCIT exhibit their efficacy have not been fully elucidated.

Despite the usefulness of SLIT, it has been reported that approximately 30% of JCP patients do not respond to this therapy. Dividing patients into high-responder (HR) and non-responder (NR) groups could be helpful in understanding the mechanisms of SLIT. We recently performed a clinical study of SLIT with cedar pollen extract on 193 adult patients with JCP. Among 142 patients who completed 2 years of SLIT, 102 (72%) showed more than 1 level of improvement in the severity score. Regardless of whether they improved due to a placebo effect, we selected the top 33 HR patients in order of improvement rank. The bottom 34 NR patients were also selected, and their serum factors were comparatively analyzed before and after SLIT. Although the HR and NR groups were not distinguishable by any single parameter, they could be clearly separated by processing the parameters with an ensemble algorithm, Adaptive Boosting. We also analyzed the population of peripheral blood CD4+ T cells, basophils, conventional dendritic cells, and plasmacytoid dendritic cells. Although there were no significant differences in these populations, between the HR and NR groups, CD4+ T cells are implicated in the effect of AIT. In addition, by using cluster analysis for all serum parameters, we found that the presence of specific cytokines for Th1 and Th2 cell subsets was strongly correlated with HR but not NR patients in our previous study. Therefore, comparative genome-wide transcriptome analyses with CD4+ T cell mRNA, isolated from the HR and NR patients, were performed herein. After the exclusion of samples of cypress pollen-specific IgE-positive patients and samples in which the RNA or DNA was damaged, 25 samples each in the HR and NR groups underwent microarray analyses. We identified 56 genes, differentially expressed between the HR and NR patients, based on the log2 ratio of their averages (Fig. 1. Among these, 5 genes encoded taste receptors, 4 of which tended to increase in the HR group but not in the NR group, after SLIT. Consistently, the expression of TAS2R13, 43 and 50 in CD4+ T cells could be retrieved by BioGPS (http://biogps.org/) (Supplementary Figs. 1–3). Among them, we confirmed the cell surface expression of TAS2R43 on CD4+ T cells (Supplementary Fig. 4). SLIT-induced increasing tendency was also observed for several small nuclear RNAs and micro-RNAs especially in the HR group. The results of one-way two-class ANOVA of the log2 ratios suggested that the pre-treatment expression level distributions of those genes were biased between the HR and NR groups.

To identify gene expression-related and germline gDNA structural variations, a genome-wide copy number variation (CNV) analysis was performed. Several CNV regions relating to differential mRNA expression between the HR and NR groups were identified (Supplementary Table 1, Supplementary Fig. 5). Figure 2A shows one such CNV region on chromosome 12 that contains several TAS2R genes. Deletion-type CNVs in this region, in a Japanese population, have also been reported previously. Genome-wide CNV and mRNA association analysis indicated a significant correlation between the CNV and mRNA expression level for the TAS2R43 gene in the HR group, but not the NR group, both before and after SLIT (Fig. 2B, C). Taste receptors are G-protein-coupled receptors located on the tongue, and are often expressed by airway smooth muscle cells and mast cells. Deshpande et al. consistently showed that TAS2R agonists such as saccharin, chloroquine, and denatonium (DN) induced the relaxation of isolated human airway smooth muscle cells. Ekoff et al. demonstrated that IgE-mediated mast cell degranulation was suppressed by TAS2R agonists. To ascertain the functional role of TAS2R2 in CD4+ T cells, the effects of TAS2R2 agonists, e.g., DN and phenylthiocarbamide (PTC), on Th2 cytokine expression were examined. Stimulation through T cell receptors and CD28 strongly induced interleukin (IL)-4, IL-5, and IL-13 mRNA expression in CD4+ T cells (Supplementary Fig. 6A), though their enhanced levels were much different among donors. Interestingly, the expression of IL-4 but not IL-5 or IL-13 was slightly but significantly augmented by the addition of DN and PTC (Supplementary Fig. 6B). Although mechanisms underlying the differential contribution of TAS2R2 to each cytokine remain to be further elucidated, these findings suggest that the difference in taste receptor expression may affect CD4+ T-cell responsiveness, and consequently, SLIT efficacy.

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Fig. 1. Differential mRNA expression in CD4\(^+\) T cells, between HR and NR patients. A genome-wide transcriptome analysis was performed for CD4\(^+\) T cells from the HR and NR groups, as described in the Supplementary Methods. Log2 ratios of the average HR and NR levels in the CD4\(^+\) T cells before (pre) and after (post) SLIT and those of the average pre- and post-treatment levels in the HR and NR groups are indicated. The genes in which the difference of the log2 ratio between the two groups was >0.1 before and after treatment are listed.
Fig. 2. CNV differences in the TAS2R43 gene, between the HR and NR group. By means of a genome-wide CNV analysis with gDNA, a differential CNV region was identified around the TAS2R43 gene. The genomic coordinates of the CNV region and the accompanying genes on both the strands in chromosome 12 are shown (A, left panel). Along with the coordinates, the marker axis shows the probe positions. The probe-level gain and loss statuses of HR and NR samples are plotted in red and blue, respectively, as the log2 ratio values (top X-axis) of the detected signals (A, right two panels). The CNV regions showing gain and loss statuses are respectively indicated with boxes in magenta and cyan and the height is scaled proportionately with the mutation rate in the population (bottom X-axis). Significantly different regions are indicated with solid lines. *P < 0.05, **P < 0.01 (N = 25, Fisher’s exact test). The correlation between TASR43 mRNA expression and CNV in the HR and NR groups before (B) and after (C) SLIT was examined. Normal distributions were confirmed by the D’Agostino–Pearson omnibus normality test.
Together with our recent findings that serum Th1/Th2 cytokines were strongly correlated in HR but not NR patients, the differences in the relationship among serum cytokines and the taste receptor expression in CD4+ T cells may be involved in the mechanisms underlying SLIT efficacy.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.alit.2018.02.003.

Conflict of interest

The authors have no conflict of interest to declare.

References


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