Needle-free Epicutaneous For t 2 DNA Vaccine is Effective for Preventing and Treating Biting Midge (*Forcipomyia taiwana*) allergy in a murine model

Mey-Fann Lee¹, Chi-Sheng Wu¹, Shyh-Jye Lin², and Yi-Hsing Chen¹

¹Taichung Veterans General Hospital

²Chung Shan Medical University

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To the Editor,

Allergen-specific immunotherapy (ASIT) remains the only treatment capable of inducing immune tolerance to the corresponding allergen and potentially treating the root cause of the allergic disease.¹ As the treatment course of protein-based vaccines for ASIT is time-consuming, an easily administered epicutaneous anti-allergic DNA-based vaccine is an attractive method, especially in light of the COVID-19 pandemic.² We established a mouse model of biting midge allergy to test the concept of the epicutaneous DNA vaccine.

The biting midge, *Forcipomyia taiwana*, is the most prevalent cause of biting insect allergy in Taiwan. It is a tiny hematophagous midge that attacks en masse. As many as 60% of exposed individuals develop allergic reactions to the bites.³ The midge is widely distributed throughout Taiwan and southern China. Among the identified allergens, For t 2 is the most predominant, with 75% of midge-allergic patients showing specific IgE to For t 2.⁴

E.coli-expressed For t 2 recombinant protein (rFor t 2) was used as an allergen to sensitize and challenge the mice.⁵ For t 2-encoding fragment (GenBank accession EU678971) was amplified by PCR. The PCR products were subcloned into pVAX1 (Life Technologies, Carlsbad, CA). The experiments were designed using two approaches: therapeutic and prophylactic (Fig 1). Twenty-five µg For t 2 DNA was determined as the optimal dose after several dose-finding experiments (Fig S1, data not shown). For each treatment, the hair of the abdominal area of the mice was removed using a depilatory, tape-stripped, then patched with 25 µg For t 2 DNA vaccine for one hour and removed. A total of three treatments were given spaced one week apart (Fig 1 and Fig S2). For t 2 proteins were detected in the patched skin and the immune organ spleen at 24 hours and had significantly increased at 48 hours (Fig S3). Scratch bouts after rFor t 2 challenge were used as a clinical surrogate of itch. We measured total IgE and For t 2-specific IgG2a in the sera as well as mRNA and proteins of IL-13, interferon-gamma, IL-10, and FOXP3 in the culture supernatants of splenocytes after stimulation with various doses of rFor t 2 at 37 for 3-5 days by ELISA and real-time quantitative PCR. Histopathology of the challenged skins was examined.

We found that after epicutaneous DNA vaccination, the allergen-induced itch of the mice significantly improved, and For t 2-specific IgG2a increased (Fig 2). Both mRNA and protein of IL-13, and eosinophils infiltration in the targeted skin, significantly decreased. Expression of FOXP3 mRNA increased (Fig S4-S6).

This is the first study to demonstrate an epicutaneous anti-allergic DNA vaccine that is effective at both treating an established allergic condition and preventing the development of an allergic disease using biting

midge allergy as a model. After epicutaneous DNA vaccination, in addition to the significant improvement in allergen-induced itch, the changes of biomarkers for allergic inflammation, including IgE, allergen-specific IgG2a, allergen-challenge-induced eosinophil infiltration in the skin, Th2 cytokines from the splenocytes, and regulatory T cell-related transcription factors, suggest that immune tolerance was induced after three patches of the epicutaneous DNA vaccine.

Our data show that though the molecular weight of the For t 2 DNA vaccine is as high as 4000 base pairs, it is able to penetrate the dermal barrier and translates the corresponding protein in the targeted skin as well as the spleen of the vaccinated mice. It is possible that the DNA vaccine passes the epidermis via the hair follicles as the skin is tape-stripped before epicutaneous vaccination.⁶

The mode of this anti-allergic epicutaneous DNA vaccine may have potential for use in other specific immunotherapies for other allergens.

Mey Fann Lee¹

Chi Sheng Wu²

Shyh Jye Lin³

Yi Hsing Chen^{2,4*}

¹Department of Medical Research, Taichung Veterans General Hospital, Taichung, Taiwan

²Division of Allergy, Immunology and Rheumatology, Taichung Veterans General Hospital, Taichung, Taiwan

³School of Medical Laboratory and Biotechnology, Chung Shan Medical University, Taichung, Taiwan

⁴School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan

Correspondence

Yi-Hsing Chen, Division of Allergy, Immunology and Rheumatology, Taichung Veterans General Hospital, 1650 Taiwan Boulevard, Section 4, Taichung 40705, Taiwan

Email ysanne@vghtc.gov.tw

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CONFLICTS OF INTEREST

The authors have no conflicts of interest with regard to this study.

Author contributions

LMF and CYH designed studies, interpreted the results and wrote the article. WCS performed experiments and analyzed data. LSJ gave technical support and revised the manuscript.

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Figure legends

Figure 1 Schedule of therapeutic (A), prophylactic (B), vaccination and grouping (C) for For t 2 DNA with skin patches. Time points of sensitization with rFor t 2/alum, videotaping, and sacrificing are indicated.

Figure 2 Scratching bouts and change in serum For t 2-specific IgG2a antibodies of mice from (A) therapeutic and (B) prophylactic groups. The scratching counts were recorded for 20 minutes after challenge with intradermal injection of rFor t 2 at the endpoints of the two experiments. The median value is marked with a black line. * $p < 0.05$, ** $p < 0.01$ by one-way analysis of variance with the Bonferroni multiple range test.

Figure S1 Dose finding schedule of For t 2 DNA patch vaccine on rFor t 2-sensitized mice in the pilot study.

Figure S2 The representative skin images show before (A) and after (B) patch application. The For t 2 DNA vaccine was delivered onto the pre-shaved abdominal skin through a Fin chamber for 1 hour.

Figure S3 H&E and immunohistochemistry (IHC) staining with rabbit anti-For t 2 polyclonal antibody of skin and spleen sections under 400x light microscope. For t 2 protein expression of skin and spleen after vaccination was examined after skin patch for 24 and 48 hours in mouse abdominal skin.

Figure S4 Effects of For t 2 vaccine on histopathology of challenged skins from (A) therapeutic or (B) prophylactic groups by H&E staining. Figures showed the representative abdominal skin sections obtained 48 hours after intradermal challenge under 100 x and 400 x light microscope. Moreover, the infiltrating inflammatory cells were quantified under 400-fold view from each group. The statistical significance of differences between groups was assessed by the Bonferroni multiple range test. ** denoted $p < 0.01$, ns denotes not statistically significant.

Figure S5 mRNA and protein expression levels of Th2 and Th1 cytokines in For t 2-stimulated splenocytes from (A) therapeutic and (B) prophylactic groups by real-time PCR and ELISA. Real-time PCR data are expressed as mean of increasing folds $\pm$ SEM. The statistical significance of differences between groups was assessed by the Bonferroni multiple range test. * denotes $p < 0.05$, ** denotes $p < 0.01$, ns denotes not statistically significant.

Figure S6 mRNA and protein expression levels of IL-10 and mRNA of the FOXP3 transcription factor in For t 2-stimulated splenocytes from (A) therapeutic or (B) prophylactic groups by real-time PCR and ELISA, respectively. Real-time PCR data are expressed as mean of increasing folds $\pm$ SEM. The statistical significance of differences between groups was assessed by the Bonferroni multiple range test. * denotes $p < 0.05$, ns denotes not statistically significant.