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REFERENCES

- Palendira U, Low C, Chan A, Hislop AD, Ho E, Phan TG, et al. Molecular pathogenesis of EBV susceptibility in XLP as revealed by analysis of female carriers with heterozygous expression of SAP. *PLoS Biol* 2011;9:e10001187.
- Ma CS, Hare NJ, Nichols KE, Dupré L, Andolfi G, Roncarolo MG, et al. Impaired humoral immunity in X-linked lymphoproliferative disease is associated with defective IL-10 production by CD4⁺ T cells. *J Clin Invest* 2005;115:1049-59.
- Kanegane H, Yang X, Zhao M, Yamato K, Inoue M, Hamamoto K, et al. Clinical features and outcome of X-linked lymphoproliferative syndrome type 1 (SAP deficiency) in Japan identified by the combination of flow cytometric assay and genetic analysis. *Pediatr Allergy Immunol* 2012;23:488-93.
- Booth C, Gilmour KC, Veys P, Gennery AR, Slatter MA, Chapel H, et al. X-linked lymphoproliferative disease due to SAP/SH2D1A deficiency: a multicenter study on the manifestations, management and outcome of the disease. *Blood* 2011;117:53-62.
- Parolini S, Bottino C, Falco M, Augugliaro R, Giliani S, Franceschini R, et al. X-linked lymphoproliferative disease: 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein-Barr virus-infected cells. *J Exp Med* 2000;192:337-46.
- Ban SA, Salzer E, Eibl MM, Linder A, Geier CB, Santos-Valente E, et al. Combined immunodeficiency evolving into predominant CD4⁺ lymphopenia caused by somatic chimerism in JAK3. *J Clin Immunol* 2014;34:941-53.

- Palendira U, Low C, Bell AI, Ma CS, Abbott RJ, Phan TG, et al. Expansion of somatically reverted memory CD8⁺ T cells in patients with X-linked lymphoproliferative disease caused by selective pressure from Epstein-Barr virus. *J Exp Med* 2012;209:913-24.
- Rivat C, Booth C, Alonso-Ferrero M, Blundell M, Sebire NJ, Thrasher AJ, et al. SAP gene transfer restores cellular and humoral immune function in a murine model of X-linked lymphoproliferative disease. *Blood* 2013;121:1073-6.
- Bollard CM, Gottschalk S, Torrano V, Diouf O, Ku S, Hazrat Y, et al. Sustained complete responses in patients with lymphoma receiving autologous cytotoxic T lymphocytes targeting Epstein-Barr virus latent membrane proteins. *J Clin Oncol* 2014;32:798-808.

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Comparison of a new Skin Prick Test Tape with the conventional skin prick test



To the Editor:

The skin prick test (SPT) has been used as a primary diagnostic tool to detect type I hypersensitivity reactions in individual patients or to screen for atopy in epidemiologic studies.¹ Studies comparing the SPT to *in vitro* test systems such as ImmunoCap and total serum IgE have shown that the SPT has the best positive predictive value and the best efficiency to diagnose respiratory atopic diseases.² Furthermore, the SPT gives immediate information for both patients and physicians. Although it is the major diagnostic test for type I immediate hypersensitivity worldwide, such as for allergic rhinitis, asthma, and occupational and food allergy, further developments concerning standardization and ease of application are needed.^{3,4} A new device, the Skin Prick Test Tape (SPT Tape), could provide a convenient, sterile, and standardized application for both patients and doctors. The SPT Tape is used once only and then discarded. SPT Tapes can be adapted in size and number of allergens; they can hold from 2 up to possibly 8 allergens and 2 control solutions (on both forearms), which can be selected per region, country, or group of allergens (inhalant, food allergens). Furthermore, if preferred, the SPT Tape can be filled by the physician with allergens for individual patient use. Advantages and disadvantages of the SPT Tape versus the SPT are listed in Table I.

The SPT Tape (international patent application PCT/EP2015/069283) has been designed to facilitate the performance of SPTs in everyday allergy diagnosis. It consists of an aluminum base foil coated with high-density polyethylene, aluminum, and polyamide, which carries 10 chambers, each holding a fleece (polymer) in a ring and 3 micro-needles (2 mm apart from each other). The adhesive tape consists of a double-sided paste layer to bond the cover foil and the base foil to each other, separating the chambers from each other.

The SPT Tape tested here consists of 4 small chambers for 1 allergen (100 μ L 10 histamine equivalent potency solution of *Dermatophagoides pteronyssinus*; Soluprick, ALK, Horsholm, Denmark) and 2 control solutions (saline and histamine 10 mg/mL); 1 chamber was left blank. The SPT Tape, using adhesive for medical purposes, was fixed to the forearm; chambers are activated one by one by applying pressure with the finger (for illustration, see Fig 1, A), pushing the allergen-contaminated needles 0.8 to 1 mm into the skin. The middle part of the SPT Tape is removed after 5 minutes; side strips

TABLE I. Advantages and disadvantages of the SPT Tape vs SPT

Advantages and disadvantages	SPT Tape	SPT
Advantages	● Sterility of the allergens, 1-time use ● Standardization of the allergen panel (international/national standards) ● Uniformity of skin penetration depth ● No need for keeping patients' arms horizontally fixed ● Ease of application for the personnel ● Sealed testing chamber, no cross- contamination between allergens ● No appearance of needles, ideal for children ● Nearly no pain (micro-needles) ● Clear labeling of allergens	● Currently used by most MDs worldwide ● Flexibility, large number of allergens available ● Relatively inexpensive
Disadvantages	● Costs may be higher than those for conventional SPT ● Removal of SPT Tape may be unpleasant in patients with hairy forearms ● Not suitable for patients with severe skin disease or those taking certain drugs such as antihistamines and β-blockers	● May cause pain for some patients ● Unintentional (interindividual) variations described ● Requires stock of allergens ● Sterility may be at risk when testing many patients with allergen solutions from the same vial ● Risk of cross-contamination during the performance of the test ● Not suitable for patients with severe skin disease or those taking certain drugs such as antihistamines and β-blockers

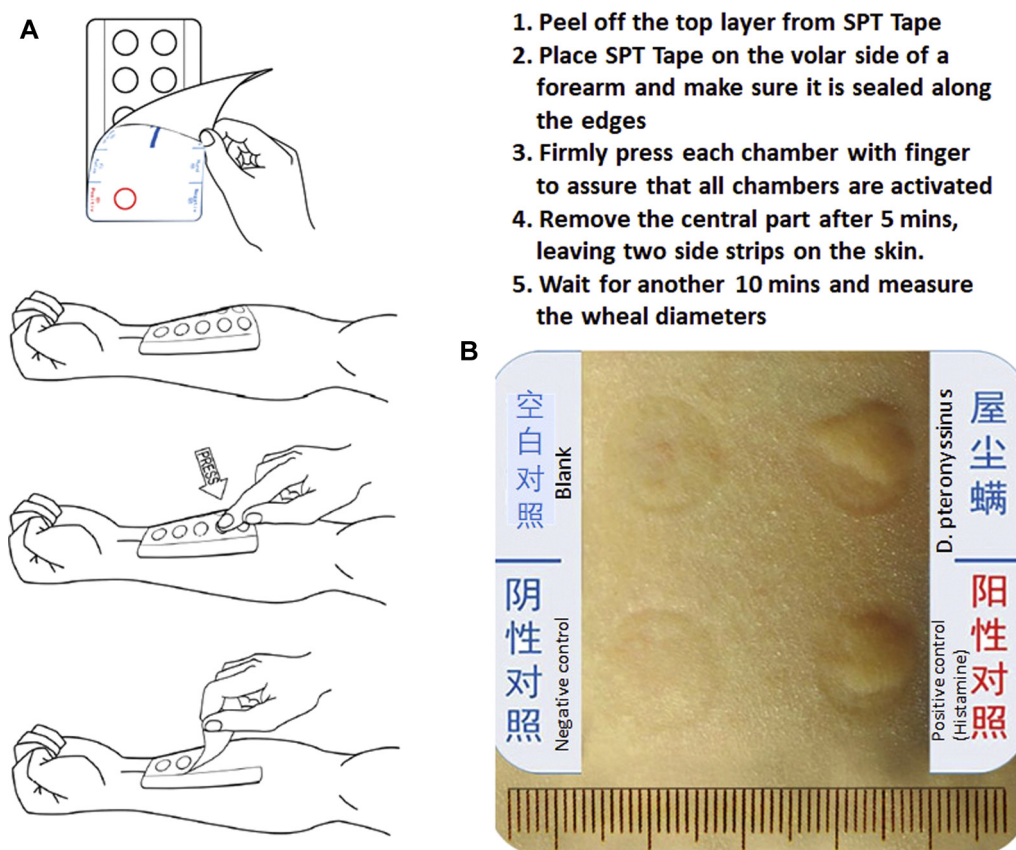


FIG 1. A, Illustration of the SPT Tape device and testing procedures. B, Wheals resulting from SPT Tape testing on the forearm of a patient.

identifying the allergen and solutions are kept in place until wheals are read after 15 minutes (Fig 1, B).

To evaluate the bioequivalence of the SPT Tape in comparison with the conventional SPT, and to compare safety and comfort of

both procedures, we enrolled 144 subjects (62% women, 38% men, mean age, 34.6 ± 12.5 years), including 72 subjects with and 72 subjects without house dust mite allergy, as evaluated by symptoms and previous allergy test results (specifically, specific

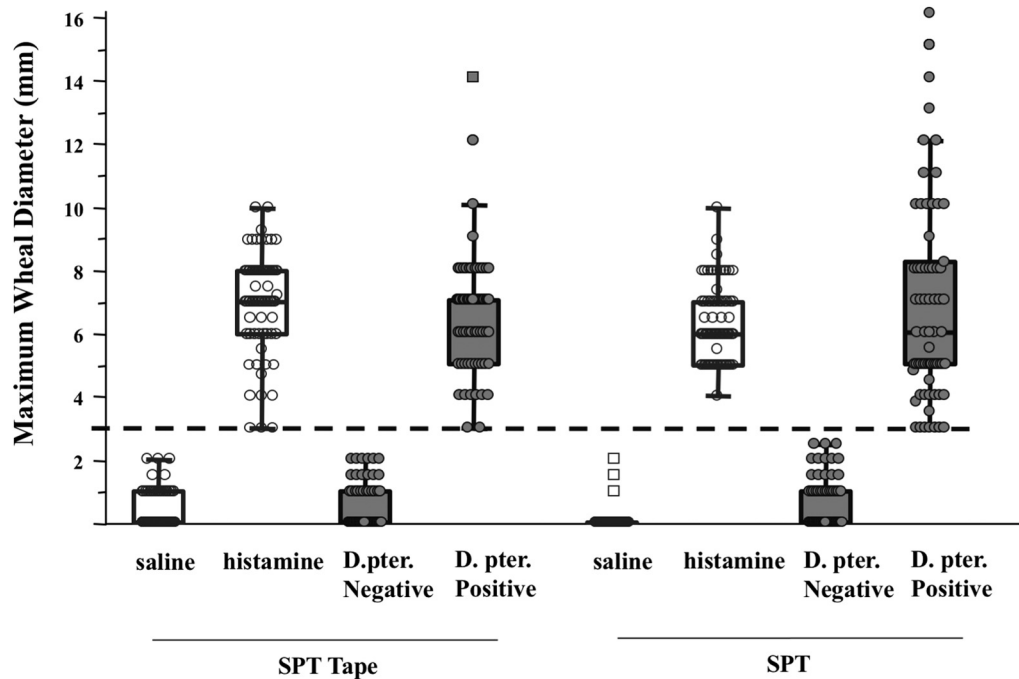


FIG 2. Wheal diameters in 144 subjects. Tested solutions were saline, histamine 10 mg/mL, and *D pteronyssinus* (*D. pter.*; ALK) in all subjects. Identical 72 subjects were positive (wheal diameter ≥ 3 mm) and 72 subjects were negative (wheal diameter < 3 mm) for *D pteronyssinus* with both diagnostic methods. The Spearman rank correlation coefficient ($r = 0.745$) between SPT Tape and SPT in detecting *D pteronyssinus* allergy was statistically significant ($P < .00$).

IgE to house dust mite [Unicap system, Thermo Fisher Scientific Inc, Uppsala, Sweden] or SPT). In all subjects, the conventional SPT was done on the left forearm according to current guidelines,^{4,5} and the SPT Tape was placed on the right forearm. Subjects were included from 2 allergy centers: The First Affiliated Hospital of Guiyang Medical College and the Medical University Affiliated Hospital of Guizhou, China. The Ethics committees of the 2 hospitals approved this study. All subjects met the inclusion criteria, voluntarily agreed to participate, and signed the informed consent. Patients taking antihistamines or other drugs interfering with the testing were excluded from the study. The SPT Tape loaded with allergens was kept at 2°C to 8°C.

The conventional SPT was performed in parallel on the same subject. After marking the place of testing for each solution with a pen on the skin, a drop each of identical positive and negative solutions as well as of the *D pteronyssinus* allergen extract was applied to the skin of the left forearm. With the help of a single-head metal lancet per drop (ALK), the skin was pricked through each drop, and test results were interpreted 15 minutes after application.

A positive SPT or SPT Tape result was defined as a wheal of greater than or equal to 3 mm longest diameter for both methods.⁶ All tests were readable and could be evaluated. All histamine SPT results were positive; all saline test results were negative and not different from the blanks. The concordance of results for the allergen (*D pteronyssinus*), calculated as the sum of positive and negative observations in agreement between the 2 methods, was 100%. The wheal diameter of the histamine reaction was about 1 mm larger with the SPT Tape (7.4 ± 1.7 mm) compared with that with the conventional SPT (6.3 ± 1.5 mm; $P < .01$; Fig 2). There were no significant differences in the wheal diameters for

the saline (0.4 ± 0.6 vs 0.3 ± 0.7 mm; $P > .05$) or the house dust mite reactions (3.6 ± 3.5 vs 3.8 ± 4.1 mm; $P > .05$).

The SPT Tape induced significantly less pain (visual analog scale [VAS] score) than the conventional SPT (0.0 [0.0-2.0] vs 2.0 [0.0-4.0]; $P < .00$); 98% of all subjects and 99% of the investigators preferred the SPT Tape over the conventional SPT. Adverse events were not recorded for both techniques; local tolerability was very good.

The SPT uses the presence and the degree of cutaneous reactivity to an allergen as a surrogate marker for the degree of sensitization within target organs,⁷ that is, nose, eyes, gut, and lung. More than 20 different allergens can be tested in parallel using both forearms, rendering the SPT a cheap, easy-to-handle, and rapid diagnostic tool. However, cross-contamination between allergens during application and testing has been noted. Repeatability of the SPT is dependent on the individual investigator, and different investigators will induce skin reactions of different sizes.⁸ And finally, sterility may be at risk when testing many patients with allergen solutions from the same vial.

The SPT Tape overcomes critical points of the conventional SPT by standardizing the application procedure, easing the reading of results (by the side strips), avoiding cross-contamination by separating allergens in different chambers, guaranteeing sterility, and finally easing the handling of the SPT for the patient, who remains free in moving around, and the physician, sparing personnel (as he or she can apply the test himself or herself within short time), room (patient can return to waiting area), and finally costs. Especially children, not any more exposed to visible needles and pain sensations, may appreciate this SPT Tape system.

We here have shown that the SPT Tape delivered bioequivalent results to the conventional SPT for the diagnosis of house dust mite allergen sensitization. The SPT Tape was significantly less painful and clearly preferred over the conventional SPT by nearly all patients. Further tests with various allergens should be performed to establish the possible role of the SPT Tape in daily allergy practice.

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REFERENCES

1. Heinzerling LM, Burbach GJ, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA(2)LEN skin test study I: GA(2)LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. *Allergy* 2009;64:1498-506.
2. Tschopp JM, Sistek D, Schindler C, Leuenberger P, Perruchoud AP, Wuthrich B, et al. Current allergic asthma and rhinitis: diagnostic efficiency of three commonly used atopic markers (IgE, skin prick tests, and Phadiatop). Results from 8329 randomized adults from the SAPALDIA Study. *Swiss Study on Air Pollution and Lung Diseases in Adults. Allergy* 1998;53:608-13.
3. Fatteh S, Rekerth DJ, Hadley JA. Skin prick/puncture testing in North America: a call for standards and consistency. *Allergy Asthma Clin Immunol* 2014;10:44.
4. Heinzerling L, Mari A, Bergmann KC, Bresciani M, Burbach G, Darow U, et al. The skin prick test – European standards. *Clin Transl Allergy* 2013;3:3.
5. Position Statement. Allergy skin testing. Board of Directors. American Academy of Allergy and Immunology. *J Allergy Clin Immunol* 1993;92:636-7.
6. Konstantinou GN, Bousquet PJ, Zuberbier T, Papadopoulos NG. The longest wheal diameter is the optimal measurement for the evaluation of skin prick tests. *Int Arch Allergy Immunol* 2010;151:343-5.
7. Bousquet J, Lebel B, Dhivert H, Bataille Y, Martinot B, Michel FB. Nasal challenge with pollen grains, skin-prick tests and specific IgE in patients with grass pollen allergy. *Clin Allergy* 1987;17:529-36.
8. Malling HJ, Allesen-Holm P, Karved LS, Poulsen LK. Proficiency testing of skin prick testers as part of a quality assurance system. *Clin Transl Allergy* 2016;21:36.

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Bcl2-like protein 12 plays a critical role in development of airway allergy through inducing aberrant T_H2 polarization



To the Editor:

Although it is well understood that skewed T_H2 polarization plays a critical role in the pathogenesis of allergic asthma,¹ the mechanism of T_H2 polarization remains elusive.² Our recent

studies showed that Bcl2-like protein 12 (Bcl2L12) was associated with pathogenesis of T_H2-biased inflammation in the intestine by facilitating T_H2 polarization.³ Thus we hypothesize that Bcl2L12 might be also associated with the pathogenesis of allergic asthma.

In this study we developed a mouse strain with Bcl2L12 knockout (KO) CD4⁺ T cells (ie, KO mice; see Fig E1 in this article's Online Repository at www.jacionline.org); KO mice and wild-type (WT) mice were sensitized to ovalbumin (OVA) to develop airway allergy according to published procedures.⁴ After sensitization, WT mice showed asthma-like inflammation in the airway, including profound infiltration of mononuclear cells in lung tissue, increase in thickness of the basal membrane of bronchial walls (Fig 1, A), antigen-specific IgE in sera, and high levels of T_H2 cytokines and mouse mast cell protease 1 in both sera and bronchoalveolar lavage (BAL) fluid, which were much lower in KO mice. The signature T_H1 cytokines IFN-γ, IL-17, and TNF were also detected in sera and BAL fluid of both WT and KO mice, although levels were lower in sensitized WT mice compared with those in control mice (Fig 1, B-E). In BAL fluid total cell, eosinophil, lymphocyte, neutrophil, and macrophage counts were significantly greater in sensitized WT mice than those in sensitized KO mice (Fig 1, F-J). Lung resistance was much greater in sensitized WT mice than in sensitized KO mice (Fig 1, K). The data suggest that Bcl2L12 plays a critical role in development of asthma-like inflammation in the airway.

BAL fluid was obtained from asthmatic patients and healthy subjects (see Table E1 for patient demographic data in this article's Online Repository at www.jacionline.org). Cellular elements were prepared from BAL fluid samples. Compared with samples from healthy subjects, BAL fluid samples from asthmatic patients showed a T_H2-dominant profile, as indicated by a higher frequency of T_H2 cells and high levels of T_H2 cytokines (Fig 2, A-C, and see Fig E2 in this article's Online Repository at www.jacionline.org). Expression of Bcl2L12 in CD4⁺ T cells isolated from BAL fluid samples was greater in the asthma group than that in the healthy group (Fig 2, D and E; see Table E2 for primers used in RT-qPCR in this article's Online Repository at www.jacionline.org). A positive correlation was identified between Bcl2L12 and IL-4 ($r = 0.7172$, $P = .0196$), IL-5 ($r = 0.7545$, $P = .0117$), and IL-13 ($r = 0.8076$, $P = .0047$) in BAL fluid CD4⁺ T cells collected from the asthma group (Fig 2, F-H). A negative correlation ($r = 0.8953$, $P = .0005$) was identified between Bcl2L12 expression in BAL fluid CD4⁺ T cells and FEV₁ of asthmatic patients (Fig 2, I). Similar phenomena were also found in peripheral blood CD4⁺ T cells of the same group of asthmatic patients (see Fig E3 in this article's Online Repository at www.jacionline.org). No correlation was detected between Bcl2L12 and IFN-γ, IL-17, or TNF (data not shown). These data imply that Bcl2L12 might be involved in the development of the skewed T_H2 response in asthmatic patients.

CD4⁺ T cells were isolated from blood samples collected from asthmatic patients and healthy subjects to gain insight into the mechanism by which Bcl2L12 regulates T_H2 response. After stimulation with activators in culture for 4 days, more IL-4⁺ T cells were induced in naive CD4⁺ T cells collected from asthmatic patients than those from healthy subjects, which was abolished by knocking down Bcl2L12 expression.