

Accepted Manuscript

Skin Treatment with Detergent Promotes Protease Allergen-Dependent Epicutaneous Sensitization in a Manner Different from Tape Stripping in Mice

Hirono Ochi, Toshiro Takai, Sakiko Shimura, Natsuko Maruyama, Izumi Nishioka, Seiji Kamijo, Hideo Iida, Susumu Nakae, Hideoki Ogawa, Ko Okumura, Shigaku Ikeda

PII: S0022-202X(17)31155-7

DOI: [10.1016/j.jid.2017.02.970](https://doi.org/10.1016/j.jid.2017.02.970)

Reference: JID 776

To appear in: *The Journal of Investigative Dermatology*

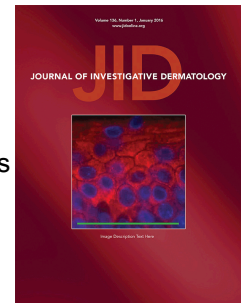
Received Date: 19 December 2016

Revised Date: 1 February 2017

Accepted Date: 15 February 2017

Please cite this article as: Ochi H, Takai T, Shimura S, Maruyama N, Nishioka I, Kamijo S, Iida H, Nakae S, Ogawa H, Okumura K, Ikeda S, Skin Treatment with Detergent Promotes Protease Allergen-Dependent Epicutaneous Sensitization in a Manner Different from Tape Stripping in Mice, *The Journal of Investigative Dermatology* (2017), doi: 10.1016/j.jid.2017.02.970.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



**Skin Treatment with Detergent Promotes Protease Allergen-Dependent
Epicutaneous Sensitization in a Manner Different from Tape Stripping in Mice**

Hirono Ochi^{1,2}, Toshiro Takai^{1,*}, Sakiko Shimura^{1,2}, Natsuko Maruyama^{1,2}, Izumi Nishioka^{1,2}, Seiji Kamijo¹, Hideo Iida^{1,2}, Susumu Nakae^{1,3,4}, Hideoki Ogawa^{1,2}, Ko Okumura¹, and Shigaku Ikeda^{1,2}

¹*Atopy (Allergy) Research Center and* ²*Department of Dermatology and Allergology, Juntendo University Graduate School of Medicine, Tokyo, Japan;* ³*Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan;* ⁴*Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency, Saitama, Japan*

***Address correspondence and reprint requests to:** Toshiro Takai,
Atopy (Allergy) Research Center, Juntendo University Graduate School of Medicine,
2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan.
Phone: +81-3-5802-1591; Fax: +81-3-3813-5512; E-mail: t-takai@juntendo.ac.jp

Abbreviations

e.c.: epicutaneous, epicutaneously

i.n.: intranasal, intranasally

SDS: sodium dodecyl sulfate also known as sodium lauryl sulfate

TSLP: thymic stromal lymphopoietin

TSLPR: TSLP receptor

KEYWORDS: Epicutaneous sensitization; Murine model; Protease allergen;

Detergent; Thymic stromal lymphopoietin; IL-33

TO THE EDITOR

Skin is considered as a major route for allergen sensitization in not only atopic dermatitis but also “atopic march”, the natural progression of allergic diseases in which development of atopic dermatitis predates that of food allergy, asthma, or allergic rhinitis (Egawa and Kabashima, 2016). Proteases contained in allergen sources are frequently allergens themselves and the protease activity has been shown to cause barrier dysfunction in the mucosal epithelium and skin and stimulate various types of cells (Takai and Ikeda, 2011). A model protease allergen, papain, induces IgE/IgG1 responses upon intranasal (i.n.) (Kamijo et al., 2013) or epicutaneous (e.c.) administration in mice. Papain can cause e.c. sensitization even when applied onto intact ear skin (Iida et al., 2014) and tape stripping of ear skin prior to the e.c. papain administration remarkably promotes dermatitis and accelerates the IgE/IgG1 responses (Shimura et al., 2016). Here, we examine the effects of another type of barrier disruption *i.e.* skin treatment with detergent, sodium dodecyl sulfate (SDS also known as sodium lauryl sulfate), mimicking barrier disruption induced by daily use of soap, shampoo, and skin/hair care products, upon e.c. papain sensitization and atopic march upon subsequent airway exposure to low-dose papain (for further information on materials and methods see **Online Methods**). Mice were used in accordance with the guidelines of the Institutional Committee on animal experiments.

Papain solution was applied onto ear lobes with or without skin SDS pretreatment (**Fig. 1a**). Papain plus the SDS pretreatment induced significant elevations in serum total IgE and papain-specific IgE/IgG1 levels (**Fig. 1b** and **Supplementary Fig. S1**). On the other hand, e.c. administration of ovalbumin did not induce antibody response with or without the skin SDS pretreatment (**Fig. 1c**). In the “SDS model”, papain induced

increased epidermal thickness and cellular infiltration into the dermis (**Fig. 1d, e**), although it was much smaller than in the “tape-stripping model”, which induced remarkable ear swelling (**Fig. 1f**). Environmental proteases including papain degrade tight junction molecules, may also loosen the stratum corneum protein structure, and can stimulate various types of cells such as epidermal keratinocytes, airway epithelial cells, basophils, mast cells, and so on (Takai and Ikeda, 2011; Nakamura et al., 2006; Kato et al., 2005; Kamijo et al., 2013; Stremnitzer et al., 2015; Seaf et al., 2016). We assume that combination of the stratum corneum barrier defect, which is induced by the pretreatment of skin with SDS or tape stripping, and the tight junction barrier defect and innate immune responses, which are induced by allergen protease, might be essential to their cooperation.

Cytokines derived from keratinocytes or epithelial cells, such as thymic stromal lymphopoietin (TSLP) and IL-33, have important roles in Th2-skewed sensitization and development of allergic diseases (Ziegler, 2012; Cayrol and Girard, 2014). IL-33-deficiency (Oboki et al., 2010) did not decrease antibody response in the e.c. sensitization phase with or without the SDS pretreatment (**Fig. 2b**) similarly to the tape-stripping model (Shimura et al., 2016). After the e.c. sensitization, mice were i.n. challenged with low-dose papain (**Fig. 2a**), which induced no significant responses in mice without e.c. presensitization to papain. I.n. challenge induced eosinophilic airway inflammation (**Fig. 2c** and **Supplementary Fig. S2**) and maintenance of papain-specific IgE levels (**Fig. 2d, middle**) in wild-type but not IL-33^{-/-} mice. Mice deficient with the receptor for TSLP (TSLPR) showed less antibody response than wild-type mice in the SDS model (**Fig. 2e**) but not in the tape-stripping model (**Fig. 2f** and **Supplementary Fig. S4**). Restimulation of skin draining lymph node cells collected after six times of e.c.

papain administration in the SDS model (**Fig. 2g**) induced increased release of Th2 cytokines, IL-5 and IL-13, in wild-type mice and it was markedly reduced in TSLPR^{-/-} mice (**Fig. 2h** and **Supplementary Fig. S5**). I.n. challenge after six times of e.c. papain administration induced severe lung eosinophilia in wild-type mice in the SDS model, which was significantly attenuated in TSLPR^{-/-} mice (**Fig. 2i** and **Supplementary Fig. S6**).

Distinct features were observed between the SDS and tape-stripping models, *i.e.* the e.c. sensitization was partially dependent on the TSLP-TSLPR pathway in the SDS model but not the tape-stripping model and dermatitis induced was much more severe in the tape-stripping model (**Figs. 1e, f** and **2e, f**). Different effects on skin homeostasis by detergent (Torma et al., 2008) and tape stripping (Nagao et al., 2012) could be causes for the different features between the two models, although mechanisms are yet to be investigated. In a model of e.c. sensitization to ovalbumin by painting it to shaved, SDS-pretreated back skin, ovalbumin-specific IgE production was almost absent in TSLPR^{-/-} mice and was not dependent on IL-33 (Muto et al., 2014). Thus, dependency on the TSLP-TSLPR pathway in e.c. sensitization to protein antigens could be variable according to the types of barrier dysfunction or adjuvant activity of stimuli, while IL-33 seems to be dispensable.

We consider that the attenuated lung eosinophilia in TSLPR^{-/-} mice (**Fig. 2i**) could be mainly due to the attenuated Th2 sensitization before i.n. challenge (**Fig. 2h**) and in part due to the lack of signaling mediated by airway-derived TSLP, because contribution of the TSLP-TSLPR pathway to atopic march induced after four times of e.c. papain administration was ambiguous (**Supplementary Fig. S3b, c**) compared to the apparent contribution of IL-33 (**Fig. 2c, d**) and TSLP contributes to an i.n. papain sensitization

model at a smaller extent than IL-33 (our unpublished observations).

In conclusion, we showed that detergent-mediated skin damage further promotes the IgE/IgG1 responses and Th2 differentiation in the e.c. sensitization to protease allergen, and that TSLP and IL-33 contribute to e.c. papain sensitization and atopic march upon subsequent airway low-dose encounter with the protease allergen, respectively (**Supplementary Fig. S7**). The SDS and tape-stripping models of e.c. papain sensitization showed distinct features, suggesting that detergent-mediated and mechanical (such as scratching-mediated) barrier damages promote protease allergen-dependent e.c. sensitization in different manners. These models would be useful for elucidation of differential mechanisms underlying e.c. sensitization towards atopic march specific to the types of barrier dysfunction and adjuvant activity, which are mediated by environmental proteases, detergent, mechanical damage, or their combinations.

Mice were maintained in a specific pathogen-free animal facility at Sankyo Labo Service Corporation (Ibaraki, Japan), were transferred to that at Juntendo University, and were used in accordance with the guidelines of the Institutional Committee on animal experiments.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Mayu Suzuki, Punyada Suchiva, Yosuke Ito, Mutsuko Hara, Rumi Ichikawa, and Liang Ning for their technical assistance, Toyoko Hidano and Takatoshi Kuhara for animal care, Michiyo Matsumoto for her secretarial assistance, Steven F. Ziegler for providing TSLPR^{-/-} mice, and Susumu Nakae and Hirohisa Saito for providing IL-33^{-/-} mice. We also thank staffs of the Laboratory of Morphology and Image Analysis of Juntendo University Graduate School of Medicine for specimen preparation for histology and Eri Hirasawa, Etsuko Komiyama, and the Gender Equality Promotion Center of Juntendo University for arrangement for the participation of R.I. and L.N. to this study. This study was supported by Ministry of Education, Culture, Sports, Science and Technology-supported Program for Strategic Research Foundation at Private Universities (S1101010), JSPS KAKENHI (JP25461711 and JP26860759), and the Takeda Science Foundation.

REFERENCES

- Cayrol C, Girard JP. IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy. *Curr Opin Immunol* 2014;31:31-7.
- Egawa G, Kabashima K. Multifactorial skin barrier deficiency and atopic dermatitis: Essential topics to prevent the atopic march. *J Allergy Clin Immunol* 2016;138:350-8 e1.
- Iida H, Takai T, Hirasawa Y, Kamijo S, Shimura S, Ochi H, et al. Epicutaneous administration of papain induces IgE and IgG responses in a cysteine protease activity-dependent manner. *Allergol Int* 2014;63:219-26.
- Kamijo S, Takeda H, Tokura T, Suzuki M, Inui K, Hara M, et al. IL-33-mediated innate response and adaptive immune cells contribute to maximum responses of protease allergen-induced allergic airway inflammation. *J Immunol* 2013;190:4489-99.
- Kato T, Takai T, Mitsuishi K, Okumura K, Ogawa H. Cystatin A inhibits IL-8 production by keratinocytes stimulated with Der p 1 and Der f 1: biochemical skin barrier against mite cysteine proteases. *J Allergy Clin Immunol* 2005;116:169-76.
- Muto T, Fukuoka A, Kabashima K, Ziegler SF, Nakanishi K, Matsushita K, et al. The role of basophils and proallergic cytokines, TSLP and IL-33, in cutaneously sensitized food allergy. *Int Immunol* 2014;26:539-49.
- Nagao K, Kobayashi T, Moro K, Ohyama M, Adachi T, Kitashima DY, et al. Stress-induced production of chemokines by hair follicles regulates the trafficking of dendritic cells in skin. *Nat Immunol* 2012;13:744-52.
- Nakamura T, Hirasawa Y, Takai T, Mitsuishi K, Okuda M, Kato T, et al. Reduction of skin barrier function by proteolytic activity of a recombinant house dust mite major allergen Der f 1. *J Invest Dermatol* 2006;126:2719-23.
- Oboki K, Ohno T, Kajiwarra N, Arae K, Morita H, Ishii A, et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci U S A* 2010;107:18581-6.
- Seaf M, Ben-Zimra M, Mankuta D, Dayan N, Levi-Schaffer F. Papain Activates Human Mast Cells to Release Proinflammatory Mediators via its Enzymatic Activity. *J Invest Dermatol* 2016;136:1523-5.
- Shimura S, Takai T, Iida H, Maruyama N, Ochi H, Kamijo S, et al. Epicutaneous allergic sensitization by cooperation between allergen protease activity and mechanical skin barrier damage in mice. *J Invest Dermatol* 2016;136:1408-17.
- Stremnitzer C, Manzano-Szalai K, Willensdorfer A, Starkl P, Pieper M, Konig P, et al. Papain Degrades Tight Junction Proteins of Human Keratinocytes In Vitro and Sensitizes C57BL/6 Mice via the Skin Independent of its Enzymatic Activity or TLR4 Activation. *J Invest Dermatol* 2015;135:1790-800.
- Takai T, Ikeda S. Barrier dysfunction caused by environmental proteases in the pathogenesis of allergic diseases. *Allergol Int* 2011;60:25-35.
- Torma H, Lindberg M, Berne B. Skin barrier disruption by sodium lauryl sulfate-exposure alters the expressions of involucrin, transglutaminase 1, profilaggrin, and kallikreins during the repair phase in human skin in vivo. *J Invest Dermatol* 2008;128:1212-9.
- Ziegler SF. Thymic stromal lymphopoietin and allergic disease. *J Allergy Clin Immunol* 2012;130:845-52.

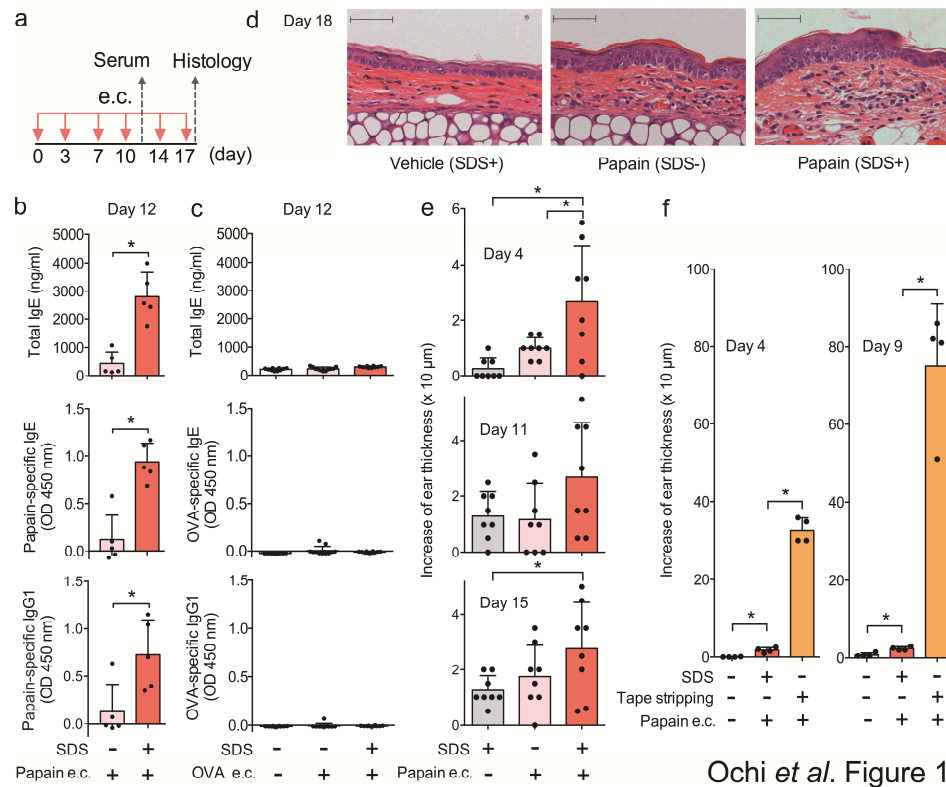
Figure Legends

Fig. 1. Skin treatment with SDS promoted epicutaneous sensitization to papain. (a)

Time line. Papain (**b, d-f**) or ovalbumin (10 mg/ml) (**c**) was administered onto the surface of both ears with or without SDS pretreatment (**b-e**) or tape stripping (**f**) in mice. (**b, c**) Serum antibodies. (**d**) Histology. (**e, f**) Ear swelling. * $P < 0.05$ by the Mann-Whitney U test (**b, c, f**) and by t -test (**e**). Data represent the mean $\pm$ SD of 5 (**b**) or 10-11 mice (**c**) or 8 (**e**) or 4 (**f**) ears per group. e.c.: epicutaneous administration. OVA: ovalbumin. Scale bar = 50 μ m.

Fig. 2. Responses in IL-33^{-/-} and TSLPR^{-/-} mice in the sensitization phase of the SDS model of epicutaneous papain sensitization and the subsequent effector phase of atopic march in airway. (a, g) Time lines (a for b-f; g for h, i). Papain (10 mg/ml)

was administered onto the surface of both ears with or without SDS pretreatment (**b-e, h, i**) or tape stripping (**f**) in mice. (**b, d-f**) Serum antibodies. (**c, i**) Eosinophilic airway inflammation after i.n. challenge with low-dose papain (2.5 μ g). (**h**) Cytokine release in restimulated skin draining cervical lymph node cells. * $P < 0.05$ by ANOVA except for **c** (t -test). # $P < 0.05$ in **d** versus before i.n. challenge (**b**) by paired t -test. ns: no significant difference in **f**. Data represent the mean $\pm$ SD of 4-6 (**b-d, f**), 4-9 (**e**), or 5-9 (**i**) mice per group or triplicate culture wells (**h**). Detection limits are indicated by dotted lines in **h**. e.c.: epicutaneous sensitization. i.n.: intranasal challenge. BAL: bronchial alveolar lavage. DLN: draining lymph node.



Ochi *et al.* Figure 1

Ochi *et al.* Figure 2

