precisely determined thus far, whereas the worldwide prevalence of EPP has been reported to be 1:75,000–1:200,000 (Todd, 1994). Thus, a nationwide survey and genotyping of the large number of Japanese EPP families is recommended and would be required to elucidate the virtual penetrance and prevalence of EPP in Japan.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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The study protocol was approved by the Ethics Committee of Hirosaki University Hospital. Informed consent was obtained from the patients described in this paper. This study was conducted according to the Declaration of Helsinki Principles.

Hajime Nakano[1], Aoi Nakano[1], Yuka Toyomaki[1], Shigeki Ohashi[1], Ken Harada[1], Ryuta Moritsugu[1], Hitoshi Takeda[1], Akira Kawada[2], Yoshihiko Mitsuhashi[3] and Katsumi Hanada[1]

[1]Department of Dermatology, Hirosaki University School of Medicine, Hirosaki, Japan; [2]Department of Dermatology, Kinki University School of Medicine, Sayama, Japan and [3]Department of Dermatology, Yamagata University School of Medicine, Yamagata, Japan.
E-mail: hnakano@cc.hirosaki-u.ac.jp

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Reduction of Skin Barrier Function by Proteolytic Activity of a Recombinant House Dust Mite Major Allergen Der f 1

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TO THE EDITOR

Exposure to house dust mite allergens is an important risk factor for the production of specific IgE and is associated with allergic diseases such as asthma, rhinitis, and atopic dermatitis (Platts-Mills and Chapman, 1987). House dust mite Group 1 allergens, Der f 1 from *Dermatophagoides farinae* and Der p 1 from *Dermatophagoides pteronyssinus*, are major allergens and belong to the papain-like cysteine protease family (Thomas *et al.*, 2002). Their proteolytic activity has been suggested to be involved in the pathogenesis of allergies by facilitating the passage of their own and other allergens across the epithelium, cleaving and/or interacting with cell-surface molecules and intrinsic protease inhibitors, and modulating the function of various cells (Comoy *et al.*, 1998; Shakib *et al.*, 1998; Gough *et al.*, 1999; Takai *et al.*, 2005a). Although mite-derived proteolytic activities have been reported to disrupt the bronchial epithelial barrier (Herbert *et al.*, 1995; Wan *et al.*, 1999), whether they disrupt the skin barrier, which is considered a much more rigid barrier system, has not been investigated. Here, we test whether the proteolytic activity of Der f 1 causes a reduction in the barrier function of the skin in nude mice using a recombinant Der f 1 (rDer f 1) with full cysteine protease activity.

We demonstrated that rDer f 1 activated with L-cysteine reduced the barrier function of the skin in dose- and time-dependent manners (Figure 1) and that the reduction was dependent on its proteolytic activity (Figure 2). All animal studies have been approved by the Review Board of Juntendo University. By the use of nude mice, experimental procedures were simplified because of their hairless phenotype, and effects of T cell-mediated acquired immunity on the barrier dysfunction could be ignored. The critical permeability function of the skin is mediated by the outermost layer of the epidermis, the stratum corneum (Strid and Strobel, 2005). The barrier function was evaluated based on two parameters, transepidermal water loss (TEWL) and the penetration by riboflavin of the stratum corneum. TEWL is a parameter for dryness of the skin, whereas riboflavin penetration is considered a parameter of the accessibility of the skin to environmental allergens and irritants. On Day 7, TEWL and riboflavin penetration were significantly increased by administration of 1 or 5 μg/site (Figure 1a) and 5 μg/site (Figure 1b) of activated rDer f 1, respectively, compared with the vehicle control. No increases were observed on Day 3 (Figure 1c and d). TEWL was greater at the patched site than water-treated site even in the vehicle control suggesting that the vehicle solution containing diluted

Abbreviations: rDer f 1, recombinant Der f 1; TEWL, transepidermal water loss

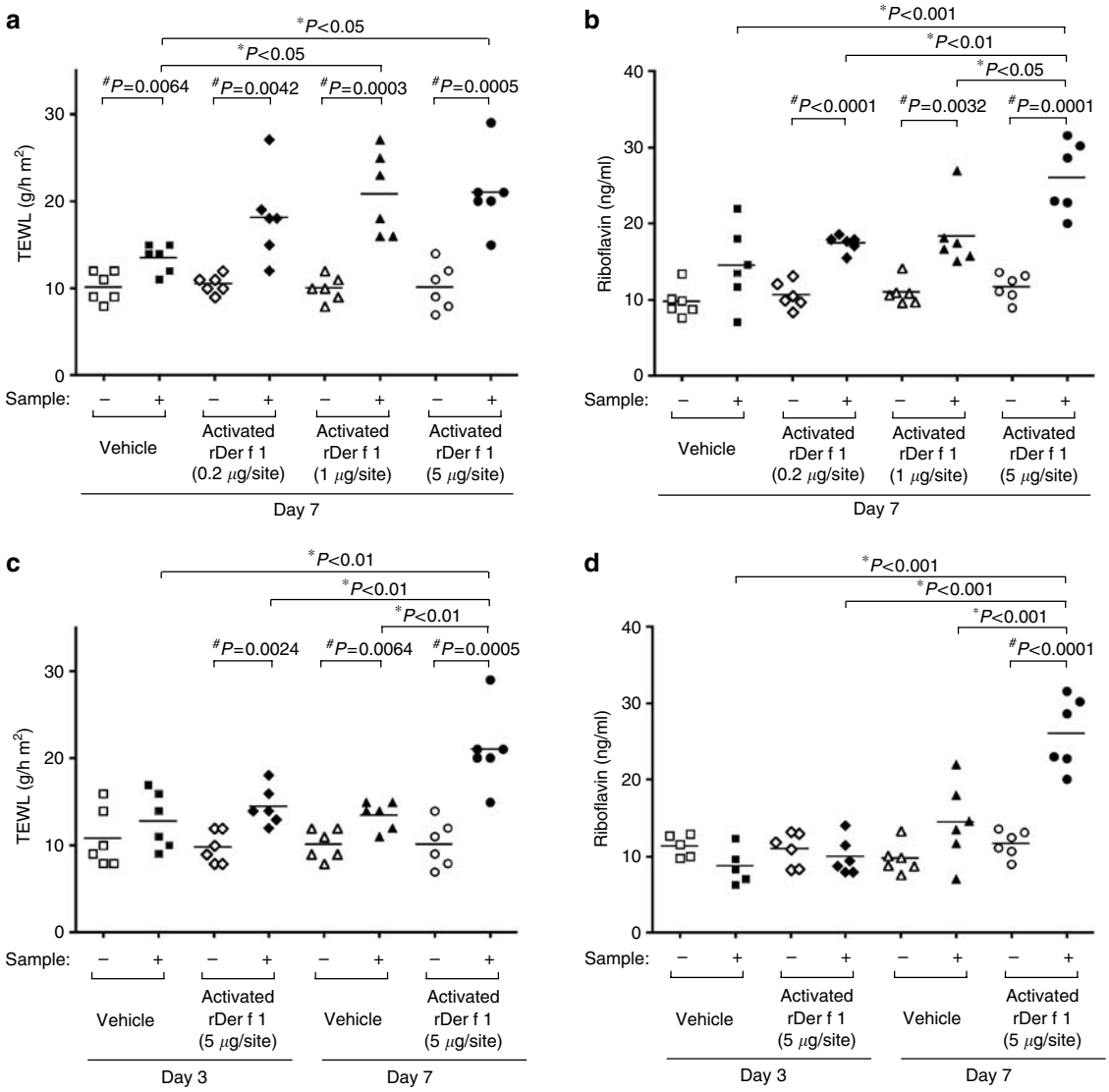

Figure 1. Dose- and time-dependent barrier dysfunction in the skin of nude mice at the sites where activated rDer f 1 was applied. Five-week-old female nude mice with the BALB/c background (Charles River Japan Inc., Yokohama, Japan) were maintained in a specific pathogen-free animal facility at Juntendo University. The glycosylated rDer f 1, designated Der f 1-WT, was expressed in yeast and prepared as described previously (Takai *et al.*, 2002, 2005b). The backs of mice anesthetized were lightly wiped with dry absorbent cotton once and cotton soaked with hexane twice. The use of hexane is to remove excess of scales. A rectangular piece of filter paper 1 cm × 2 cm (3MM Chr, Whatmann, Middlesex, UK) was placed on the back, and 100 μl of solution containing rDer f 1 was perfused to the paper. The rDer f 1 was preincubated with 1.4 mM L-cysteine and diluted with phosphate-buffered saline and pure water. The final concentrations of rDer f 1 and L-cysteine were 50, 10, or 2 μg/ml (5, 1, or 0.2 μg/100 μl/site) and 50 μM, respectively, and the final dilution of phosphate-buffered saline was 13/100. The paper was wrapped with poly(vinylidene chloride) film cut to a size of 2 cm × 3 cm and then immobilized with an adhesive bandage (KINO SELF; Nitto Denko Co., Osaka, Japan). Solutions containing rDer f 1 activated with L-cysteine or the vehicle only was applied to mice (+). Another site towards the tail away from the sample-loaded site was loaded with pure water (−) and treated as like the sample-loaded site. The solutions for loading were administered a total of four times (Day 0, 2, 4, and 6) or twice (Day 0 and 2) onto the patch every other day. The (**a** and **b**) dose and (**c** and **d**) time influenced the levels of the barrier dysfunction. (**a** and **c**) TEWL. On the day after the last administration (Day 7 or 3), the bandage, Saran Wrap, and paper were removed and the back of each mouse was lightly wiped once with absorbent cotton soaked with hexane, and then TEWL was measured with a Mobile Tewameter (Courage + Khazaka electronic GmbH, Köln, Germany). (**b** and **d**) Riboflavin test. After the measurement of TEWL, the amount of riboflavin to penetrate the stratum corneum was measured as described previously (Okuda *et al.*, 2002) with some modifications as follows. Cotton soaked with 100 μg/ml of riboflavin was placed on the site for 1 min and then removed, and the excess residual solution at the site was soaked up with wringed cotton. After drying for approximately 30 minutes, the stratum corneum was stripped with adhesive cellophane tape. The stripping was performed six times with the tape changed each time. The pieces of tape containing the stripped stratum corneum were dipped in 3 ml of a 1% sodium dodecyl sulfate solution overnight at 4°C protected from any light. The next day, the riboflavin was extracted from the tape by sonication. After vigorous mixing, the solutions were passed through a 0.2-μm filter and moved to new tubes. The concentration of riboflavin was determined by measuring the fluorescence of riboflavin. The data shown represent the values for six or five mice. Bars indicate means. The Tukey's *post hoc* test followed by one-way analysis of variance was used to evaluate the significance of the differences among the sites treated with the samples (+) ($^*P<0.05$). Unpaired Student's *t* test (two-tailed) was used to evaluate the significance of the differences between the sites treated with each sample (+) and pure water (−) ($^\#P< 0.05$). A value of $P<0.05$ was regarded as statistically significant.