Modulation of Atopy Patch Test and Skin Prick Test by Pretreatment with 1% Pimecrolimus Cream

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Key Words
Atopic eczema · Atopy patch test · Pimecrolimus

Abstract
Background: In a subgroup of patients with atopic eczema (AE), aeroallergens are relevant eliciting factors. The atopy patch test (APT) was proposed as inflammation model for AE. ***Objective:*** It was the aim of this study to investigate the effect of pretreatment with 1% pimecrolimus cream (Elidel®) on the APT and skin prick test (SPT). ***Methods:*** In a randomized, controlled, double-blind study, 20 patients with AE and positive SPT and APT screening reaction to house dust mite *Dermatophagoides pteronyssinus,* cat dander, grass or birch pollen were enrolled (age 20 ± 11 years, 55% males). For 2 weeks, patients twice daily applied pimecrolimus and vehicle control to marked fields on their backs and forearms. Then, APT was performed (200 index of reactivity/g extracts in petrolatum; Stallergènes, France) on both fields on the back and SPT was performed on the pretreated forearms. ***Results:*** Including only patients with different readings (n = 13), stronger APT suppression of at least 1 ETFAD (European Task Force on Atopic Dermatitis) grade in the pimecrolimus area versus intraindividual control was observed in 10 of these patients after 48 and 72 h ($p < 0.05$; 90% CI 50.5–93.4). Including all 20 subjects, the analysis still showed a borderline significance compared with the vehicle ($p = 0.0564$). SPT with histamine and aeroallergens showed a median 7.5–10% reduction in actively pretreated areas ($p = 0.086$). Immunohistochemical analysis in 2 patients revealed an induction of interferon-γ in primecrolimus-pretreated skin. ***Conclusion:*** APT can be used as a model for AE skin inflammation. It was shown for the first time that pimecrolimus pretreatment has a potential to suppress the development of lesions induced by aeroallergen exposure in patients with AE.

Introduction

Atopic eczema (AE), a chronic inflammatory skin disease, is characterized by an age-related distribution and morphology and often appears together with allergic rhinitis and bronchial asthma [1, 2]. Many trigger factors like irritants, microbial organisms, stress and allergens may influence the course of AE. Aeroallergens play an important role in inducing eczema flares [3, 4]. They are able to penetrate the disturbed skin barrier [5] where they become bound to Langerhans cells and presented to T cells [6].

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Table 1. ETFAD grading of APT reactions [3]

Score		Description
–	0	negative
±	0.5	only erythema, not distinguishing allergic from irritative factors
+	1	infiltrated erythema
++	2	erythema, some papules (≤ 3), possible infiltration
+++	3	erythema, papules (≥ 4)
++++	4	erythema, many papules or spreading papules
+++++	5	erythema, (papules and) vesicles

Relevant aeroallergens can be identified by the atopy patch test (APT), an epicutaneous patch test which uses immunoglobulin (Ig)E-inducing allergens from e.g. house dust mite or cat dander, with evaluation of resulting eczematous skin reaction after 24–72 h [3]. The first systematic investigations with house dust mite allergen in patients with AE were published in 1982 by Mitchell et al. [7]. The production of Th1- and Th2-type cytokines and accumulation of allergen-specific T cells play an important role in the pathogenesis of APT reaction [8].

Pimecrolimus (SDZ ASM 981), an ascomycin derivative, is an anti-inflammatory and immunomodulatory macrolactam. It blocks T-cell activation, inhibits the synthesis of inflammatory cytokines – interleukin (IL)-2, IL-3, IL-4, IL-5, IL-10 and tumor necrosis factor-α – and prevents the release of cytokines from mast cells by inhibition of the phosphatase calcineurin [9].

The aim of this study was to evaluate a possible effect of pimecrolimus on the intensity of the APT and the skin prick test (SPT) reaction in patients with AE.

Study Design and Methods

Twenty patients (11 males, 9 females, aged 19–61 years, mean age 30.5 years) with a history of AE [2], but in remission at the time of the study, were investigated in a single-center, randomized, double-blind, intraindividual vehicle-controlled study. Before enrollment in the study, all patients gave their informed consent, and the study was approved by the appropriate ethical committee of the Technical University of Munich.

The study consisted of three periods: the screening, treatment and evaluation period.

In the screening period (days 1–14), four allergen preparations from house dust mite *(Dermatophagoides pteronyssinus),* cat dander, grass pollen and birch pollen (200 index of reactivity/g in petrolatum; Stallergènes, France) were applied in 12-mm-diameter aluminum Finn chambers (Epitest Ltd., Oy, Finland) to untreated, clinically uninvolved skin on the back. The Finn chambers were removed after 48 h and the APT was read after 48 and 72 h (days 3 and 4) according to the ETFAD (European Task Force on Atopic Dermatitis) criteria [3] (table 1). An SPT was performed on the forearm on day 1, with the same four aeroallergens (Allergopharma, Reinbek, Germany) and saline as well as 0.1% histamine as negative and positive controls. The test was read after 20 min. SPT with a wheal ≥ 3 mm was defined as positive. After 10 to maximum 30 days, the patient was included in the treatment period provided that there was at least one positive reaction in the APT in the screening phase.

During the treatment period (duration of treatment 14 ± 1 day), the patient or an assigned person had to apply pimecrolimus 1% cream (Novartis Pharma, Nuremberg, Germany) and placebo (vehicle cream) on two marked areas (about 5 cm^2) on the back and one marked area on each forearm twice daily. In addition, a third area on the back was marked but remained untreated.

In the evaluation period (days 29–32), the allergen which had shown the strongest positive reaction in the screening period – concerning the APT and SPT – was used. The APT and SPT were performed on the marked area on the back and the forearms, respectively. The reading of the APT was performed in a blinded manner.

In 2 patients who consented, a skin biopsy was taken 48 h after application of the APT.

Serum concentrations of IgE-antibodies (antigen specific and total) were determined using CAP-RAST-FEIA (Pharmacia, Uppsala, Sweden). Specific IgE antibodies (against the same aeroallergens as in the APT) with more than 0.35 kU/l (CAP class 1) were defined as positive. Blood was taken on day 15 (visit 4) and day 32 (visit 7) of the study.

Immunohistochemical Analysis

Punch biopsy specimens (5 mm) were taken after induction of local anesthesia (1% lidocaine) from the center of the patch test area. Frozen 4- to 5-μm skin sections were mounted on slides coated with poly-*L*-lysine (Sigma, Munich, Germany) and fixed with dry acetone for 30 min. The staining was done by the Tech Mate Horizon staining machine (Dako, Hamburg, Germany) with the following primary monoclonal mouse antibodies incubating for 1 h: CD1a (clone NA1/34; Dako), CD3 (polyclonal; Dako), CD4 (clone EDU-2; Novo Castra, Dossenheim, Germany), CD8 (clone C8/144B; Dako) and IgE (clone CIA-E-7.12; Dako). The alkaline phosphatase reaction was visualized using a detection kit (alkaline phosphatase red, rabbit mouse; Dako Chem Mate). Finally, the sections were embedded in gelatin.

The cytokine single staining was done manually: the sections were incubated with paraformaldehyde (Sigma) for 20 min, washed in Trizma base buffer (Sigma) containing 1% bovine serum albumin (Sigma) and then incubated with the primary antibody, i.e. interferon (IFN)-γ and IL-5 from Alexis, diluted in antibody diluent (Dako) for 1 h. The biotinylated secondary antibody was added for 25 min. After repeated washing, alkaline phosphatase was pipetted for 25 min. The reactivity of this enzyme was visualized using a detection kit (super sensitive detection kit; Bio Genex). The activity of the alkaline phosphatase was inhibited by levamisole (Dako). Finally, the sections were slightly stained with hemalaun and embedded in gelatin (Merck, Haar, Germany).

Three 4-μm sections of each biopsy were stained for each antibody and subsequently quantified with the software programme

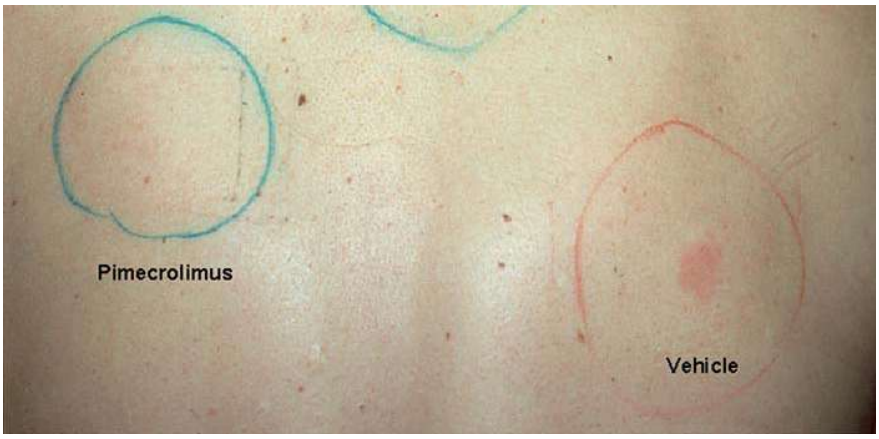

Fig. 1. APT reaction after pretreatment with pimecrolimus 1% cream and vehicle control on the back after 72 h.

Table 2. Primary endpoint analysis: influence of pimecrolimus 1% cream pretreatment on APT reactions after 48 and 72 h

Time of evaluation, h	Patients	Lower APT score at pimecrolimus area	90% confidence interval	p value
48	13	10 (76.9)	50.5–93.4	0.0461
72	13	10 (76.9)	50.5–93.4	0.0461

Only patients with different readings were included in the analysis. Figures in parentheses are percentages.

KS300 developed by Zeiss (Jena, Germany) which calculates the positive area by measuring color (in our case, red) intensity. Quantification was done in a blinded manner by 2 independent analyzers.

Statistical Analysis

In the binominal test analysis of the primary endpoint, only patients with different readings (n = 13) were included. Intraindividual differences in APT and SPT reactions in active versus control sites were analyzed using the two-sided Wilcoxon signed rank test. Differences are presented together with 95% confidence intervals and associated p values. Correlations were calculated by Spearman's correlation coefficient. A 5% significance level was used throughout.

Results

All 20 screened patients were treated and completed the study.

In the screening period, the strongest test reactions were found for house dust mite in 13/20 (65%), cat dander in 2/20 (10%) and birch pollen in 5/20 (25%) patients for the APT and for house dust mite in 6/20 (30%), cat dander in 2/20 (10%), birch pollen in 6/20 (30%) and grass in 6/20 (30%) patients for the SPT.

In the evaluation period, all 20 patients again showed a positive APT reaction (reproducibility 100%). A complete suppression with pimecrolimus was seen in 3/20 (15%) of the patients. Thirteen of 20 (65%) patients showed a different APT reaction comparing vehicle- and pimecrolimus-treated areas. In 10/20 (50%) patients, pimecrolimus areas showed a lower reaction intensity than vehicle areas (borderline significance, p = 0.0564). In the other 10/20 (50%) patients, 7 patients had no difference in APT reaction between both test areas and in 3 patients the area pretreated with pimecrolimus was read with a higher ETFAD key intensity than the vehicle area. Referring to the different reactions in the APT (predefined primary study endpoint evaluation), 10/13 (76.9%) patients had a lower APT reaction in the pimecrolimus test area compared with the vehicle test area for both 48- and 72-hour readings. The 90% confidence interval was 50.5–93.4, and the p value was 0.0461 for both time points (table 2). An example is shown in figure 1.

There was a trend, but no significant difference, between the SPT reactions on areas pretreated with pimecrolimus and the vehicle (p = 0.0856): SPT with histamine and aeroallergens showed a median 7.5–10% reduction in actively pretreated areas. Differences in SPT reactions were measured by diameter of wheals.

Allergen-specific APT (*D. pteronyssinus* and cat dander) results were significantly correlated with corresponding specific serum IgE levels (fig. 2).

Correlations between SPT and specific serum IgE levels were as follows: r = 0.72, p = 0.003 for cat dander; r = 0.65, p = 0.002 for house dust mite; r = 0.58, p = 0.03 for birch pollen.

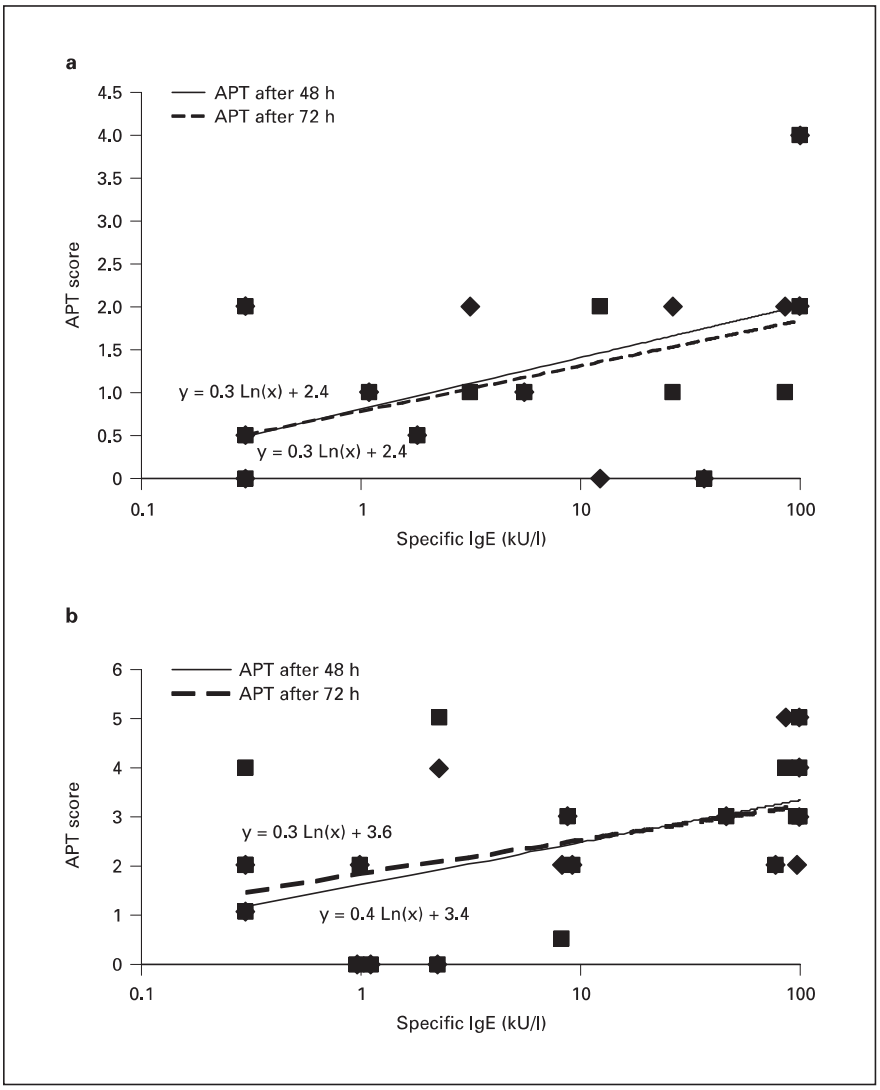

Fig. 2. Correlations calculated by Spearman's correlation coefficient between APT reaction and serum IgE concentrations. Individual values of each patient after 48 (◆) and 72 h (■). **a** Regression lines of allergen cat dander ($r = 0.48$, $p < 0.05$ for APT after 72 h). **b** Regression lines of allergen house dust mite ($r = 0.66$, $p = 0.001$ for APT after 48 h and $r = 0.51$, $p = 0.02$ for APT after 72 h).

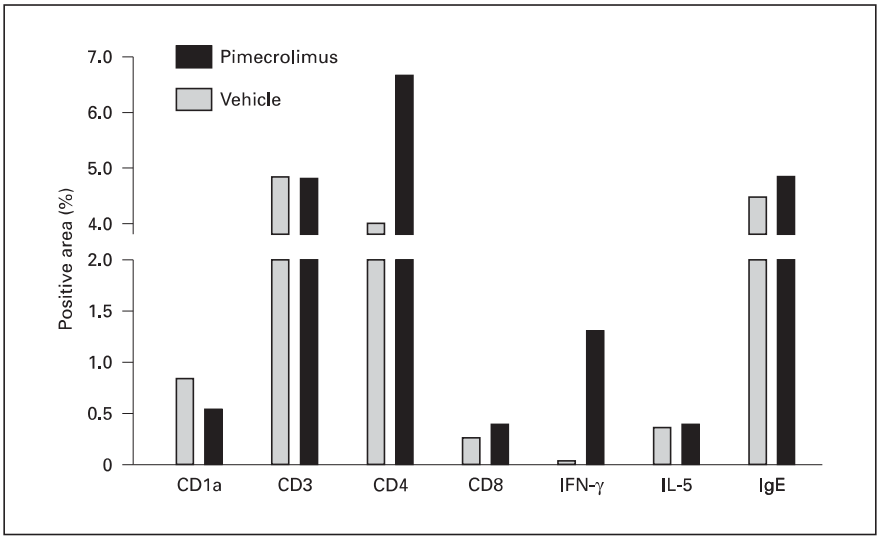

Fig. 3. Cytokine expression in skin biopsies of APT sites pretreated with pimecrolimus or vehicle after 48 h.

Safety

No serious adverse events were seen. The study procedure was well tolerated. No local side effects due to pimecrolimus were seen.

Immunhistochemical Analysis

Biopsies of the vehicle- and pimecrolimus-pretreated areas were taken after 48 h of patch testing in 2 patients, analyzed by immunhistochemistry and subsequently quantified. APT reactions showed a strong influx of mononuclear cells, the majority being CD3- and CD4-positive T cells, whereas CD8-positive T cells were sparse. CD4-positive T cells increased in the pimecrolimus-pretreated area, whereas CD8 and CD3 markers remained unchanged. Concerning the cytokine profile of the patch tests, a clear enhancement of IFN-γ-producing T cells occurred after the pretreatment of the skin with pimecrolimus. CD1a exhibited a lower expression after pretreatment with pimecrolimus (fig. 3).

Discussion

This study demonstrated that pretreatment with the topical calcineurin inhibitor pimecrolimus was able to decrease the intensity of the APT reaction. Pimecrolimus was developed for the treatment of inflammatory skin diseases and was recently approved for use in patients with mild to moderate AE. It is reported to be suitable for the long-term management of eczema and for reducing or eliminating flares and the need for corticosteroid treatment [10, 11]. It is well tolerated and does not cause steroid-associated local effects, such as dermal atrophy, striae or telangiectasia [10, 11].

While treatment of eczematous skin lesions is easy, the prevention is difficult. There are only few studies using the APT as a model where topical pretreatment demonstrated a prophylactic effect. Billmann-Eberwein et al. [12] found that the use of fatty acid-rich emollients can prevent or diminish the development of APT reactions. These reactions were mostly only erythematous lesions without infiltration. Pretreatment of APT areas with topical glucocorticosteroids and tar could significantly reduce allergic inflammation in patients with AE [13].

The SPT reaction was tendentiously reduced by 2 weeks of pretreatment with pimecrolimus in contrast to the study of Spergel et al. [14] who showed that 2 weeks of pretreatment with pimecrolimus could not diminish the SPT reaction compared with a vehicle control. The mechanism underlying this observation may be an inhibitory effect on mast cells [9], yet the clinical relevance of the use of pimecrolimus in the immediate-type hypersensitivity is unclear.

Hultsch et al. [15] have demonstrated that pimecrolimus inhibits the release of granule-associated mediators and newly synthesized cytokines in RBL 2H3 mast cells in an immunophilin-dependent manner. In 1999, Greiding and Moreno [16] showed that doxepin incorporated into a cream emollient had antipruritic effects and inhibited wheal size in SPT – presumably by its antihistamine effect.

Immunohistochemical analysis of APT biopsies revealed a reduction in epidermal dendritic cells (CD1a+) after pimecrolimus pretreatment. These results are in line with the recently described reduction in CD1a+/CD11b+ cells after tacrolimus treatment in AE [17]. Interestingly, CD4+ cells were more prominent in pimecrolimus-pretreated skin while CD8+ cells – already being sparse – remained unchanged. Expression of IFN-γ was enhanced after pimecrolimus pretreatment, suggesting an immunomodulating effect of pimecrolimus in the direction of a Th1 profile. IgE-mediated effects seemed to be unaffected by pimecrolimus.

This is the first study investigating the modulation of APT as a model for AE by pimecrolimus. Pimecrolimus seems to be able to act against aeroallergen-induced AE flares in susceptible patients, possibly through its inhibitory effect on T-cell activation. Further studies are necessary to elucidate the potential of pimecrolimus in the tertiary prevention of eczematous flares in AE and to figure out the characteristics of patient subgroups with the highest benefit of a prophylactic pretreatment with pimecrolimus. This may even lead to a disease-modifying effect as aeroallergen-induced eczematous lesions coincide with a defective skin barrier and may facilitate further allergen penetration through the skin [18].

Acknowledgments

We thank Dr. Rita Tanke for monitoring the study and Johanna Grosch and Simone Knabl for the laboratory assistance.

References

1 Ring J: Atopy: condition, disease or syndrome? In Ruzicka T, Ring J, Przybilla B (eds): Handbook of Atopic Eczema. Berlin, Springer, 1993, pp 3–8.

2 Hanifin JM: Basic and clinical aspects of atopic dermatitis. Ann Allergy 1984;52:386–395.

3 Darsow U, Ring J: Airborne and dietary allergens in atopic eczema: a comprehensive review of diagnostic tests. Clin Exp Dermatol 2000; 25:544–551.

4 Morren MA, Przybilla B, Bamelis M, Heykants B, Reynaers A, Degreef H: Atopic dermatitis: triggering factors. J Am Acad Dermatol 1994; 31:467–473.

5 Gondo A, Saeki N, Tokuda Y: Challenge reactions in atopic dermatitis after percutaneous entry of mite antigen. Br J Dermatol 1986;115: 485–493.

6 Maurer D, Ebner C, Reininger B, Fiebiger E, Kraft D, Kinet JP, et al: The high affinity IgE receptor mediates IgE-dependent allergen presentation. J Immunol 1995;154:6285–6290.

7 Mitchell EB, Crow J, Chapman MD, Jouhal SS, Pope FM, Platts-Mills TA: Basophils in allergen-induced patch test sites in atopic dermatitis. Lancet 1982;1:127–130.

8 Grewe M, Walther S, Gyufko K, Czech W, Schopf E, Krutmann J: Analysis of the cytokine pattern expressed in situ in inhalant allergen patch test reactions of atopic dermatitis patients. J Invest Dermatol 1995;105:407–410.

9 Zuberbier T, Chong SU, Grunow K, Guhl S, Welker P, Grassberger M, Henz BM: The ascomycin macrolactam pimecrolimus (Elidel, SDZ ASM 981) is a potent inhibitor of mediator release from human dermal mast cells and peripheral blood basophils. J Allergy Clin Immunol 2001;108:275–280.

10 Bernard LA, Bergman JN, Eichenfield LF: Pimecrolimus 1% cream (Elidel) for atopic dermatitis. Skin Therapy Lett 2002;7:1–3.

11 Eichenfield LF, Beck L: Elidel (pimecrolimus) cream 1%: a nonsteroidal topical agent for the treatment of atopic dermatitis. J Allergy Clin Immunol 2003;111:1153–1168.

12 Billmann-Eberwein C, Rippke F, Ruzicka T, Krutmann J: Modulation of atopy patch test reactions by topical treatment of human skin with a fatty acid-rich emollient. Skin Pharmacol Appl Skin Physiol 2002;15:100–104.

13 Langeveld-Wildschut EG, Riedl H, Thepen T, Bihari IC, Bruijnzeel PL, Bruijnzeel-Koomen CA: Modulation of the atopy patch test reaction by topical corticosteroids and tar. J Allergy Clin Immunol 2000;106:737–743.

14 Spergel JM, Nurse N, Taylor P, ParneixSpake A: Effect of topical pimecrolimus on epicutaneous skin testing. J Allergy Clin Immunol 2004;114:695–697.

15 Hultsch T, Müller KD, Meingassner JG, Grassberger M, Schopf RE, Knop J: Ascomycin macrolactam derivative SDZ ASM 981 inhibits the release of granule associated mediators and of newly synthesized cytokines in RBL 2H3 mast cells in an immunophilin-dependent manner. Arch Derm Res 1998;290:501–507.

16 Greiding L, Moreno P: Doxepin incorporated into a dermatologic cream: an assessment of both doxepin antipruritic action and doxepin action as an inhibitor of papules, in allergen and histamine-caused pruritus. Allergol Immunopathol (Madr) 1999;27:265–270.

17 Schuller E, Oppel T, Bornhovd E, Wetzel S, Wollenberg A: Tacrolimus ointment causes inflammatory dendritic epidermal cell depletion but no Langerhans cell apoptosis in patients with atopic dermatitis. J Allergy Clin Immunol 2004;114:137–143.

18 Gfesser M, Rakoski J, Ring J: The disturbance of epidermal barrier function in atopy patch test reactions in atopic eczema. Br J Dermatol 1996;135:560–565.