

Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy

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Background: Subcutaneous allergen-specific immunotherapy is an effective treatment of IgE-mediated allergies, but it requires repeated allergen injections with a risk of systemic allergic reactions. Transcutaneous immunotherapy may improve patient compliance and safety.

Objective: To assess the safety and efficacy of epicutaneous allergen immunotherapy.

Methods: This monocentric, placebo-controlled, double-blind trial was conducted from March 2006 to December 2007 at the University Hospital Zurich. Thirty-seven adult patients with positive skin prick and nasal provocation tests to grass pollen were randomized to receive patches containing either allergen (n = 21) or placebo (n = 16). Treatment took place before and during the pollen season 2006, and follow-up visits took place before (n = 26) and after the pollen season 2007 (n = 30). The primary outcome measures were nasal provocation tests.

Results: Allergen-treated patients showed significantly decreased scores in nasal provocation tests in the first ($P < .001$) and second year ($P = .003$) after treatment. In contrast, placebo-treated patients had decreased scores in the first treatment year, 2006 ($P = .03$), but the effect diminished in the second year ($P = .53$). Although improvement of nasal provocation test scores was not significantly better in the verum versus placebo group, the overall treatment success was rated significantly higher by the allergen-treated group than by the placebo group (2006, $P = .02$; 2007, $P = .005$). No severe adverse events were observed. Occurrence of eczema after allergen patch applications proved stimulation of specific T-cell responses, but was noted as an adverse effect of the treatment.

Conclusion: Epicutaneous allergen immunotherapy is a promising strategy to treat allergies and merits further investigation. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Allergen-specific immunotherapy, patch, grass pollen allergy, rhinoconjunctivitis, randomized clinical trial

IgE-mediated allergies affect up to 35% of the population in westernized countries.¹ The only disease-modifying and causal treatment is specific immunotherapy, which is typically performed by subcutaneous administration of gradually increasing allergen doses. Once a maintenance dose is reached, it is given at monthly intervals for 3 to 5 years. The allergen injections may cause allergic reactions, and anaphylaxis is a major risk of specific immunotherapy,² so specific immunotherapy must be given under the close supervision of trained physicians.

The clinical effectiveness of specific immunotherapy is well established by controlled prospective studies.^{3,4} Although the immunologic mechanisms responsible for ameliorating allergy symptoms are still a matter of debate,^{5,6} immunotherapy induces prolonged clinical improvement accompanied by a persistent alteration in immunologic reactivity.⁷

Despite these benefits of immunotherapy, only a low percentage of patients with allergy prefers immunotherapy to symptomatic treatment with antihistamines and corticosteroids.⁸ To increase attractiveness and patient compliance, noninvasive specific immunotherapy regimens have been proposed, such as sublingual and oral allergen administration.⁹⁻¹¹ We propose needle-free and painless epicutaneous immunotherapy. The epidermis has a high density of professional antigen-presenting Langerhans cells. Because the epidermis is not vascularized, epicutaneous immunotherapy should carry less risk of systemic allergen distribution and systemic allergic side effects.

Although transcutaneous vaccination has long been considered an alternative to conventional subcutaneous vaccination against childhood diseases and other infections,^{12,13} no studies have so far explored the potential of patches as a dosage form for allergen-specific immunotherapy.

METHODS

Study population

Study subjects were recruited by advertising in local newspapers. Eighty patients called the clinics for a first telephone interview, during which they were informed about the details of the study and questioned for inclusion and exclusion criteria. Thirty-nine patients came to the clinics for screening, and 37 patients fulfilled the following inclusion and exclusion criteria: age 18 to 65 years, history of summer hay fever for more than 2 seasons, and positive reactions to grass pollen allergen extract in nasal provocation and skin prick tests (*Phleum pratense*). Exclusion criteria were atopic eczema; perennial allergic rhinitis; infection of the upper airways within the last 2 weeks; surgical intervention within the last 30 days; history of HIV/AIDS; malignancy; mastocytosis; uncontrolled high blood pressure; pregnancy or lactation; presence or history of significant cardiovascular, renal, pulmonary, liver, infectious,

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This study was investigator-initiated, and the allergen patch was manufactured by the hospital pharmacy. However, a patent that belongs to the University of Zurich has been licensed by a company (Allergy Innovations, Munich, Germany), which then partially funded the trial under a clinical trial agreement with the University of Zurich. Funding was independent of the study outcome.

Disclosure of potential conflict of interest: G. Senti and T. M. Kündig have received research support from Medanz Medical GmbH and the Swiss National Science Foundation. The rest of the authors have declared that they have no conflict of interest. Received for publication November 7, 2008; revised June 24, 2009; accepted for publication July 10, 2009.

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0091-6749/\$36.00

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doi:10.1016/j.jaci.2009.07.019

Abbreviations used

NPT: Nasal provocation test

SQ-U: Standardized quality units

hematologic, autoimmune, neurologic, and psychiatric disease; moderate to severe asthma; intake of antihistamines within the last 2 weeks or systemic or topical steroids within the last 5 days; intake of contraindicated medications for specific immunotherapy such as β -blockers or angiotensin-converting enzyme/angiotensin II receptor antagonists within the last week; and participation in another clinical trial within the last 60 days.

Study design

This study was a monocentric phase I/II randomized, placebo-controlled, double-blind trial to evaluate the safety and clinical efficacy of a novel epicutaneous route for specific allergen immunotherapy. The trial design is outlined in Table I. On the basis of block randomization, patients were assigned to receive either epicutaneous administration of grass pollen allergen ($n = 21$) or placebo ($n = 16$). The patches were supplied with patient numbers according to the allocation sequence that was assigned by the pharmacist with help of a random number table. The code was revealed to the researchers only after recruitment and data collection were complete.

One month before the start of the pollen season 2006, one group of patients received 4 allergen-containing patches to apply at weekly intervals. A second group received 4 placebo patches. Whereas the first patch was applied at the clinic, the following 3 patches were applied at home. Before application, the chosen area of the upper arm or shoulder was tape-stripped 6 times (TesaFilm tape, Tesa, Hamburg, Germany). Such tape stripping not only enhances penetration into the skin by removing part of the corneal layer but also activates keratinocytes, as described in the Discussion. The patches were left on the skin for 48 hours. During the early period of the pollen season, the patients attended the clinic for a second visit. The skin was inspected by a dermatologist. Then, 8 more patches were applied at home at weekly intervals.

Clinical endpoints were assessed before, at the beginning of, and after the pollen season of 2006. Patients attended follow-up visits before and after the pollen season of 2007. The primary outcome measure was the change in the allergic response assessed by nasal provocation tests (NPTs). Secondary outcome measures included questionnaires describing hay fever symptoms by means of a visual analog scale, the use of medication, and the occurrence of local reactions and adverse events. The main objective was to assess the safety and efficacy of epicutaneous allergen immunotherapy.

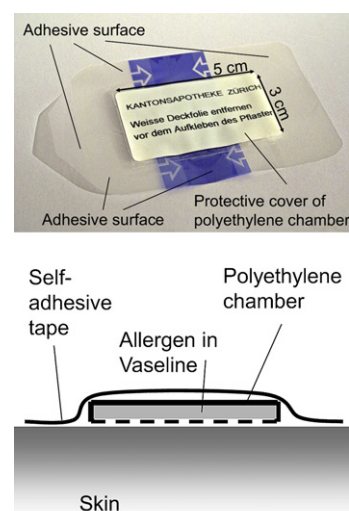
The study was performed from March 2006 to December 2007 at the University Hospital Zurich. It was reviewed and approved by the local ethics committee, and the regulatory agency, Swissmedic, was notified. Written informed consent was obtained from all patients before enrollment. The study was conducted according to International Conference of Harmonization of Good Clinical Practice guidelines and the Declaration of Helsinki.

Test drug

The patch, developed and produced by the Zurich University Hospital Pharmacy (Zürich, Switzerland) (Fig 1), consisted of a 15 cm² polyethylene pouch filled with 1.5 mL Vaseline (Vaseline PhEur, Kantonsapotheke Zürich, Switzerland) containing a total of 300 Index of Reactivity (IR) *P pratense* extract (Stallergènes, Antony, France). One side of the pouch was perforated and covered with a removable adhesive foil, imprinted with user instructions. The pouch was centered in an adhesive 6 × 9 cm Hydrofilm (Paul Hartmann Corp, Heidenheim, Germany). To guarantee 48-hour adhesion to the skin, the Hydrofilm was covered with an additional OpSite Flexifix transparent film (Smith & Nephew, Schenefeld, Germany). The patch was left on the upper arm for 48 hours. This is the same duration and allergen concentration that is used in the atopy patch test, in which Langerhans cells are known to be pulsed with protein allergens, with the only difference that the area is 15 cm² versus the 1 cm² typically used in the atopy patch test.¹⁴

TABLE I. Outline of the trial design

Visit/time	Intervention/analysis
February-March 2006	Newspaper advertisement and telephone interviews
Visit 1 (March-April 2006)	NPT, application of first patch
March-May 2006	Weekly application of next 3 patches by the patients at home
Visit 2 (April-May 2006)	Application of patch, skin inspection for erythema and edema, recording of adverse events and medication
April-September 2006	Weekly recording of symptoms, weekly application of 8 patches by the patients at home
Visit 3 (September-October 2006)	NPT, recording of improvement/deterioration on a visual analog scale, recording of adverse events and medication
Visit 4 (April 2007)	NPT
April-September 2007	Weekly recording of symptoms
Visit 5 (October-December 2007)	NPT, recording of improvement/deterioration on a visual analog scale, recording of medication

**FIG 1.** The patch consisted of a 3 × 5 cm polyethylene pouch that was filled with grass pollen extract in Vaseline. The side of the pouch that faced the skin was perforated. The pouch was held onto the skin by a self-adhesive tape.**NPT**

Counter indications for testing were episodes of rhinitis in the last 4 weeks, nasal surgery in the last 8 weeks, vaccinations within the last week, or the use of local or systemic antiallergic drugs. The patients were adapted to room temperature for 10 minutes, and anterior rhinoscopy was performed to determine baseline values. Unspecific hyperresponsiveness was excluded by administration of 50 μ L of the allergen diluent. Then, 50 μ L of the isotonic test solutions was administered to the middle nose conch by using a pipette. Subsequently, the patients were challenged with 100, 1000, 10,000 and 100,000 standardized quality units (SQ-U) of the aqueous grass pollen extract (*P pratense*, Aquagen SQ; ALK-Abelló, Volketswil, Switzerland) at 10-minute intervals. The severity of the symptoms was recorded on a visual analog scale after each allergen dose.

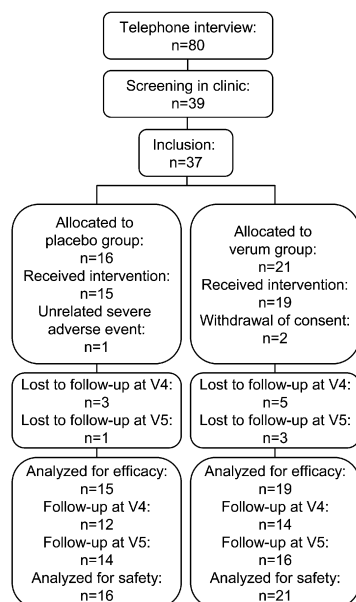


FIG 2. Participant flow. Flowchart characterizing the study population. V4, visit 4; V5, visit 5.

Skin prick test

A total of 30 μ L Soluprick SQ Grass Pollen *P pratense* 10 Histamine Equivalent Prick (HEP) Units (ALK-Abelló) was applied to the volar forearm, using histamine hydrochloride 1 mg/mL as a positive control and the diluent as a negative control.

Subjective symptom score

Patients were asked to rate the improvement or deterioration of their symptoms after the pollen seasons of 2006 and 2007 compared with the pollen season of 2005. The recording was done on a 100-mm scale without marked intervals.¹⁵

Rescue medication

During the period of immunotherapy, patients were allowed to use concomitant medicines to alleviate symptoms. The daily use of the following drugs was recorded: antiallergic eye drops (Spersallerg [Novartis, Basel, Switzerland], Zaditen Ophta [Novartis], Spersapolyomyxin [OmniVision, Neuhäusen, Switzerland], Opticrom [Sanofi-Aventis, Meyrin, Switzerland], Cromosol Ophta [Sandoz, Steinhausen, Switzerland]), antihistamines (Aerius [Essex, Luzern, Switzerland], Xyzal [UCB Pharma, Bulle, Switzerland], Telfast [Sanofi-Aventis], Claritine [Essex], Zyrtec [UCB Pharma]), antiallergic nasal sprays (Nasonex [Essex], Livostin [Janssen-Cilag, Baar, Switzerland]), topical steroids (Elocom [Essex], Diprolen Crème [Essex]), and asthma inhalers (Symbicort [AstraZeneca, Zug, Switzerland], Ventolin [GlaxoSmith-Kline, Münchenbuchsee, Switzerland]).

Adverse events

According to International Conference of Harmonization of Good Clinical Practice guidelines, serious adverse events were defined as any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect.

Eczema reaction under the patch

At the second visit, the skin was inspected by a clinician for the following symptoms: erythema, edema/induration/papule formation, excoriation,

TABLE II. Baseline characteristics by treatment group (n = 37)

Characteristics	Treatment group	
	Placebo (n = 16)	Allergen-specific immunotherapy (n = 21)
Age (y), mean $\pm$ SD	32 $\pm$ 10.6	35 $\pm$ 10.1
Female, n (%)	9 (56.3)	10 (47.6)
Weight (kg), mean $\pm$ SD	66.6 $\pm$ 11.0	67.7 $\pm$ 11.3
Height (cm), mean $\pm$ SD	172.8 $\pm$ 10.2	170.7 $\pm$ 9.4
Median score in NPT at concentration 100,000 SQ-U	5.5	5.6

TABLE III. Overview of adverse events

Adverse event	No. of adverse events/no. of patients	
	Placebo (n = 16)	Allergen-specific immunotherapy (n = 21)
Eczema under the patch	23/5	160/15
Rhinitis	4/1	3/2
Conjunctivitis	4/2	3/2
Sneezing	4/2	
Redness, swelling, wetness of upper arm		12/1
Pruritic and inflamed mamilla		3/1
Wheal		1/1
Vertigo		1/1
Prickle in arms and hands		1/1
Asthma		1/1
Scars		1/1
Total	35/5	186/16

weeping/exudation/scab formation, desquamation, and lichenification. Each of these 6 symptoms was recorded as none, 0 points; mild, 1 point; moderate, 2 points; and severe, 3 points, and a sum score was calculated.

Statistical analysis

The study was designed to detect changes from baseline (intragroup) and changes between the allergen and the placebo group in symptom scores of NPTs. An increase of the threshold dose in NPTs of 0.8 (log 10) or greater from the baseline ranks and a difference of 0.9 (log 10) between allergen treatment and placebo were considered efficacious. Thus, a sample size of 35 (allergen-specific immunotherapy, 20; placebo, 15) was required to yield a statistically significant result with a power of 80% (2-sided analysis with $\alpha = 0.05$). Comparisons between groups were calculated either with the exact 2-tailed Wilcoxon signed-rank test for related samples or with the exact Mann-Whitney *U* test for independent samples.

RESULTS

Patient characteristics

From March to April 2006, a total of 37 patients were enrolled and started treatment in the grass pollen season of 2006. Three patients discontinued treatment because of withdrawal of consent. Thus, 34 patients completed the treatment and were included in the efficacy analysis. Eight patients could not be located for the first follow-up visit in April 2007, and 4 study subjects were unable to come to the second follow-up visit between September and December 2007 (Fig 2). The demographic characteristics of the 2 treatment groups were similar at baseline (Table II).

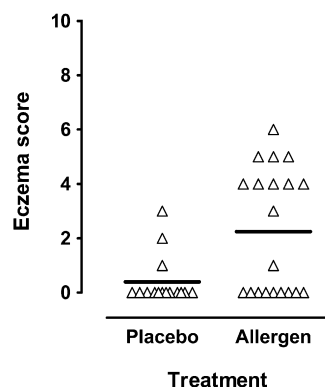


FIG 3. Local eczema reactions. At visit 2, each of 6 symptoms (erythema, edema/induration/ papule formation, excoriation, weeping/exudation/scab formation, desquamation, lichenification) was recorded as none, mild, moderate, or severe, and a sum score (0-18 points) was calculated. Verum patients (*open triangles*) showed significantly more local reactions than placebo patients (*filled triangles*; Mann-Whitney exact test, $P = .03$).

Safety and tolerability

During the entire study period, 21 (56.8%) of 37 study patients reported a total of 221 adverse events with possible relationship to study treatment. Of these 21 patients, 5 had received placebo treatment and reported a total of 35 adverse events, whereas 16 verum patients reported 186 adverse events (Table III). No serious adverse events were observed during the study.

Systemic adverse events. Rhinitis and conjunctivitis were both reported, with 3 events in the allergen group and 4 events in the placebo group. Four events of sneezing were reported in the placebo group, whereas 12 events of redness, swelling, or wetness of the upper arm and 3 events of pruritic and inflamed mamilla were reported in the allergen-treated group. The remaining adverse events were reported with 1 single event in the allergen-treated group: wheal, vertigo, prickle in arms and hands, and asthma. Because these were single events, it remains unclear whether these were symptoms of a systemic allergic reaction.

Local adverse events. The tape stripping procedure was painless and in most patients induced no visible changes of the skin. Slight irritation with erythema of the skin was the exception. The most frequent adverse events were eczemas developing at the site of patch application (allergen group, 15 patients with 160 adverse events; placebo group, 5 patients with 23 adverse events). The incidence of eczema increased with application of the first 4 patches. At the second visit, after application of the first 4 patches, the skin was inspected by a clinician. As illustrated in Fig 3, patients who received allergen-containing patches showed significantly more local reactions than the placebo group ($P = .03$). If present, these reactions were mild and ranged from 1 to 6 on a scale ranging from 0 to 18. Ten patients used topical corticosteroids for a total duration of 13 ± 20 days for the 12 patches. When asked at the final visit, patients did not report a further increase of eczema after the fourth patch. One patient developed 2 little scars where the patch was applied.

Nasal provocation

Scores for the 10,000, 1000, and 100 SQ-U provocation doses were too low to determine a threshold shift (data not shown). Using 100,000 SQ-U, the analysis of the tolerance to nasal

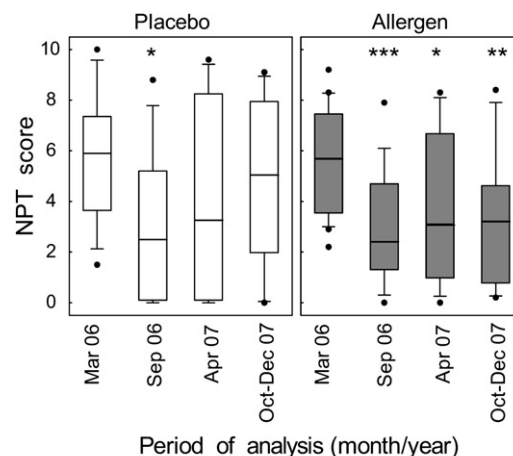


FIG 4. NPTs. Scores using the allergen dose 100,000 SQ-U are depicted. The *box plots* show the median; the 10th, 25th, 75th, and 90th percentiles; and outliers. *White box plots* represent the placebo group, and *gray box plots* represent the allergen-treated group. Statistically significant P values are indicated where values differ from baseline (* $P < .05$; ** $P < .01$; *** $P < .001$).

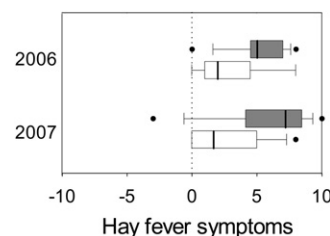


FIG 5. Patient-recorded hay fever symptoms. Patients compared symptom severity during seasons 2006 and 2007 (posttreatment) with 2005 (pretreatment) and recorded scores between -10 (worst possible deterioration) and +10 (best possible improvement). Patients receiving verum (*filled boxes*) reported better improvement than placebo patients (*open boxes*; 2006, $P = .02$; 2007, $P = .005$). *Box plots* show median; 10th, 25th, 75th, and 90th percentiles; and outliers.

allergen provocation revealed that both placebo and allergen treatment increased allergen tolerance when comparing the recorded NPT scores at baseline with those recorded approximately 3 months after the first pollen season (placebo, $P = .03$; allergen-specific immunotherapy, $P < .001$). Although the effect of allergen treatment lasted throughout the second season (2007), that of the placebo vanished when comparing baseline NPT scores to those recorded before (placebo, $P = .24$; allergen-specific immunotherapy, $P = .03$) and approximately 6 months after the pollen season of the second year (placebo, $P = .53$; allergen-specific immunotherapy, $P = .003$; Fig 4). Although the placebo effect had a trend to vanish over time, whereas the verum group stayed improved, the differences between the 2 groups did not reach statistical significance (September 2006, $P = .66$; April 2007, $P = .55$; October-December 2007, $P = .15$).

Subjective symptom scores

Patients who received allergen treatment reported a markedly stronger symptom improvement than the placebo group. As illustrated in Fig 5, the improvement of hay fever symptoms after the treatment compared with pretreatment symptoms was rated

significantly higher by patients who received allergen-containing patches than by those receiving the placebo treatment. This was observed both after the pollen season of 2006 ($P = .02$) and after the pollen season of 2007 ($P = .005$). The grass pollen load measured by the Swiss national weather service (MeteoSwiss) and the climate were not significantly different in these 2 years (data not shown).

Use of rescue medication

The use of rescue medication against hay fever was similar in the 2 test groups, attesting to the fact that both groups still had remaining hay fever symptoms, but symptoms were significantly better controlled in the verum group. Patients receiving verum, however, used significantly more topical corticosteroids to treat the eczema under the patch than the placebo group ($P = .04$). Whereas none of the placebo-treated patients used topical steroids, 10 patients from the allergen-treated group (47.6%) applied topical steroids.

DISCUSSION

The fact that the prevalence of IgE-mediated allergies has reached epidemic proportions in industrialized countries generates a strong medical need for therapy.¹ Subcutaneous immunotherapy is effective, but takes 3 years, is time-consuming, and carries a significant risk of systemic allergic reactions, including anaphylaxis.² These disadvantages impede treatment compliance.⁸

Recently, there has been increased interest in noninvasive routes for vaccine delivery such as via the skin,¹⁶⁻²² especially because of the high density²³ of potent antigen-presenting cells in the epidermis—that is, Langerhans cells.^{24,25} Because the epidermis is not vascularized, epicutaneous allergen administration could reduce the risk of allergen leaking into the circulation and provoking systemic allergic reactions.

The clinical efficacy of epicutaneous allergen administration was already suggested in the 1950s, when the French allergologist Blamoutier performed allergen-specific immunotherapy by needle scarification of the volar forearm in an area 4×4 cm.²⁶ A few minutes after dropping pollen extract onto the scarified area, Blamoutier observed the formation of a wheal-like plate, and a majority of the treated patients experienced improvement or complete relief from hay fever for 3 days to 3 weeks.²⁷ These early clinical studies were neither blind nor placebo-controlled, and they were performed at a time when immunology was in its infancy and IgE had not yet been discovered. Today, however, the likely mechanism behind his positive clinical observations is that scratching enhances allergen penetration into the epidermis and physically activates keratinocytes so that allergen-pulsed Langerhans cells leave the epidermis and migrate into regional lymph nodes for stimulation of lymphocytes. In the current study, we replaced the scarification with the adhesive tape stripping method. This procedure gently removes the cornified layers of the epidermis so that the basal membrane is not damaged and allergen penetration into the dermis is reduced.

Tape stripping of the skin induces a bystander effect mediated by keratinocyte-derived proinflammatory cytokines, an effect that cannot readily be achieved in sublingual immunotherapy. The strength and efficacy of antigen presentation and lymphocyte stimulation strongly depend on these bystander reactions, inducing secretion of IL-1, IL-6, IL-8, and TNF- α .²⁸⁻³¹ It is also reported that

tape stripping can induce production of IL-12 and IFN- γ in the skin³² and increase expression of MHC class II, CD86, CD40, CD54, and CD11c on Langerhans cells,³³ as well as enhanced expression of Toll-like receptor 9 in keratinocytes,³⁴ producing an environment favoring induction of allergy-protective immune responses mediated by Langerhans cells.^{28,35}

Although recorded as an adverse effect of the therapy, the frequent occurrence of eczema, which was observed in 71% of the allergen-treated group with 160 events, clearly suggests that the epicutaneous immunotherapy successfully pulsed and activated Langerhans cells for antigen presentation. Moreover, the fact that this eczema increased with the number of patch applications also demonstrated that allergen-specific T cells had been boosted. Consequently, patients treated with the allergen patch showed an increased tolerance in the NPTs, and this amelioration lasted for at least 1 year. In contrast, patients receiving a placebo patch showed less eczema (31% of the patients and only a total of 23 events) followed by a reduction in NPT scores during the first pollen season, but this placebo effect on allergen tolerance vanished in the second pollen season.

No patient withdrew consent to further study participation because of eczema under the patch. However, although not a threat to patient safety, follow-up studies should also be designed to reduce this potentially annoying adverse effect. This may be achieved by conducting dose-finding studies and by shortening the patch application time. A shorter application may still be sufficient to pulse the Langerhans cells, but at a later time, when T-cells start infiltrating the skin, the amount of allergen would already be reduced.

The limitations of this study were the following:

1. Most patches were applied at home, making quality control of the correct application difficult.
2. Because 8 patches were applied during the pollen season, a clear distinction between hay fever symptoms and systemic allergic reactions was more difficult than for the first 4 patches applied before the pollen season.
3. The fact that patients had to score the improvements of their actual symptoms compared with those of previous pollen seasons may contain a recall bias, which should, however, be the same for both placebo and verum patients.
4. Because grass pollen patches induced eczema in contrast with the placebo patches, one may argue that blinding of the study was limited. Occurrence of eczema, however, had no influence on study outcome, because those verum patients with eczema at the application site showed no significantly better improvement ($48\% \pm 20\%$) than those verum patients without eczema ($57\% \pm 23\%$).
5. At visit 3, a sample size of 35 (allergen-specific immunotherapy, 20; placebo, 15) would have been required to yield a statistically significant result with a power of 80% (2-sided analysis with $\alpha = 0.05$). Although the sample size at visit 3 was only 1 patient fewer than required—that is, 34, patient numbers at visits 4 and 5 were only 26 and 30, respectively.
6. Based on the NPT, we could not find a difference between placebo and verum, although with the placebo effect diminishing over time, there was a trend ($P = .15$). However, based on symptom scores during the pollen season, the amelioration in verum versus placebo patients was significantly better (2006, $P = .02$; 2007, $P = .005$).

As demonstrated for subcutaneous immunotherapy,⁷ we expect symptom amelioration to last longer than the 1½ years of observation time in this study, but longer studies will be necessary to evaluate the longevity of the treatment effect.

In conclusion, this first trial in human beings suggested that epicutaneous allergen immunotherapy of patients with rhinoconjunctivitis was safe, was well tolerated, and caused significantly stronger symptom amelioration than placebo. We assume that epicutaneous immunotherapy could also be used to treat other IgE-mediated allergies. Because it is needle-free and can be applied at home, epicutaneous allergen administration may represent a promising alternative to other methods of immunotherapy.

We thank Dr Martin Gerber for statistical analyses.

Clinical implications: Epicutaneous allergen immunotherapy may be an effective and causative treatment against IgE-mediated allergies that is safe and encourages patient compliance.

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