

REVIEW ARTICLE

Epicutaneous allergen administration: is this the future of allergen-specific immunotherapy?

G. Senti¹, S. von Moos¹ & T. M. Kündig²

¹Clinical Trials Center; ²Department of Dermatology, University Hospital of Zürich, Zürich, Switzerland

To cite this article: Senti G, von Moos S, Kündig TM. Epicutaneous allergen administration: is this the future of allergen-specific immunotherapy? *Allergy* 2011; DOI: 10.1111/j.1398-9995.2011.02560.x.

Keywords

epicutaneous allergen-specific immunotherapy; epicutaneous immunization; skin immunization.

Correspondence

Thomas M. Kündig, Department of Dermatology, Zürich University Hospital, Gloriatrasse 31, 8091 Zürich, Switzerland.
Tel.: +41 44 255 3973
Fax: +41 44 255 44 18
E-mail: thomas.kuendig@usz.ch

Accepted for publication 18 January 2011

DOI:10.1111/j.1398-9995.2011.02560.x

Edited by: Thomas Bieber

Abstract

IgE-mediated allergies, such as allergic rhinoconjunctivitis and asthma, have become highly prevalent, today affecting up to 30% of the population in industrialized countries. Allergen-specific immunotherapy (SIT) either subcutaneously or via the sublingual route is effective, but only few patients (<5%) choose immunotherapy, as treatment takes several years and because allergen administrations are associated with local and, in some cases, even systemic allergic side-effects because of allergen accidentally reaching the circulation. In order to resolve these two major drawbacks, the ideal application site of SIT should have two characteristics. First, it should contain a high number of potent antigen-presenting cells to enhance efficacy and shorten treatment duration. Secondly, it should be nonvascularized in order to minimize inadvertent systemic distribution of the allergen and therefore systemic allergic side-effects. The epidermis, a nonvascularized multilayer epithelium, that contains high numbers of potent antigen-presenting Langerhans cells (LC) could therefore be an interesting administration route. The present review will discuss the immunological rationale, history and actual clinical experience with epicutaneous allergen-specific immunotherapy.

The prevalence of allergic diseases, first described by John Bostock at the beginning of the 19th century as '*catarrhus aestivus*' (1), has been continuously increasing (2). Reaching a prevalence of up to 30% in industrialized countries, IgE-mediated allergies have become 'the new epidemics of advanced civilization'. Symptomatic treatment including antihistamines, corticosteroids and inhaled β_2 -adrenoreceptor agonists can efficiently ameliorate IgE-mediated symptoms (3). However, the only disease-modifying treatment is SIT (3, 4). Introduced a century ago by Leonard Noon and John Freeman in 1911

(5), the immunological mechanisms leading to symptom amelioration are still a matter of debate. Nevertheless, the original perception of SIT being a treatment conferring active immunity against pollen toxin (5) has changed. Nowadays, SIT is perceived as a treatment restoring normal immunity against allergens through redirection of inappropriate T-helper (Th) 2 responses (4, 6). SIT favours the production of Th1 cytokines such as interferon- γ over Th2 cytokines and induces the secretion of IL-10 and transforming growth factor- β by functional regulatory T (Treg) cells. Additionally, successful treatment is associated with the increased production of allergen-specific antibodies, especially IgG4 and to lesser extent IgA. These changes are accompanied by the suppression of mast cells, eosinophils and basophils (4, 6).

Despite the paradigm change regarding the aetiological understanding of allergy – moving from a pollen-toxin-induced disease (5) to a IgE-mediated disease caused by an inappropriate Th2-biased immune response towards innocuous environmental antigens – the clinical practice of SIT has not substantially changed since its first application by Noon and Freeman: 'Patients received subcutaneous injections of pollen extract. At first very minute doses were given...' (5).

Abbreviations

APC, antigen-presenting cell; CT, cholera toxin; DC, dendritic cell; EDS, epidermal delivery system; EPIT, epicutaneous allergen-specific immunotherapy; IL, interleukin; ILIT, intralymphatic allergen-specific immunotherapy; i.m., intramuscular; LC, Langerhans cell; LT, heat-labile enterotoxin; SALT, skin-associated lymphoid tissue; SCIT, subcutaneous allergen-specific immunotherapy; s.c., subcutaneous; SIT, allergen-specific immunotherapy; SLIT, sublingual allergen-specific immunotherapy; TCI, transcutaneous allergen-specific immunotherapy; Th, helper T cell; Treg, regulatory T cell.

Conventional SIT still consists of subcutaneous administration of gradually increasing doses of allergen (7). The need for up to 50–80 subcutaneous injections over 3–5 years and the associated risk of systemic allergic side-effects (7) limit broad patient acceptance of subcutaneous allergen-specific immunotherapy (SCIT) (8, 9). In view of these limitations, there have been several attempts during the last century (i) to improve efficacy of SIT as to reduce treatment duration, (ii) to increase safety and (iii) to offer more patient-convenient treatment routes.

The first major improvement of SIT was achieved in the 1930s when allergy vaccines were adjuvanted with Alum. Alum not only increased the immunogenicity of the vaccine but also reduced the risk of systemic allergic side-effects because of its depot effect at the injection site (10). While novel adjuvants, such as monophosphoryl lipid A (11) and CpG (12), are being developed, Alum remains the predominant adjuvant in SIT. In the 1960s, attempts were made to modify the allergen extracts in order to reduce allergenic side-effects. Hence, allergoids, i.e. chemically modified allergens with reduced IgE-binding capacity, are currently the basis of many allergy vaccines (13), and recombinant 'hypoallergenic allergens' are being developed (14). Besides these attempts to improve immunogenicity and reduce side-effects of SIT, considerable effort has been put into the development of more patient-convenient treatment administration routes. Sublingual allergen-specific immunotherapy (SLIT), which will be also reviewed in this issue, offers a needle-free and self-administrable treatment option (15), which has been recommended by the WHO in 1998. However, treatment duration is not reduced and local, i.e. oral allergic side-effects are frequent (9). Intralymphatic allergen-specific immunotherapy (ILIT), which directly delivers the antigen into organized lymphoid tissue, has been demonstrated to substantially shorten treatment duration, while at the same time, the allergen doses can be lowered, and thereby, the risk of systemic allergic side-effects is reduced (16, 17). Epicutaneous allergen-specific immunotherapy (EPIT) offers a novel, needle-free and self-administrable treatment route. In this review, we discuss the immunological rationale, history and current experience with EPIT.

The skin

Anatomical structure

Human skin is composed of two compartments: the epidermis and the dermis. The epidermis, which forms a 50- to 150- μ m thick protection layer (18), mainly consists of keratinocytes; gradually maturing from undifferentiated epidermal cells, which form the *stratum basale*, they continuously divide and differentiate to build up the stratified epidermis with the *stratum spinosum*, the *stratum granulosum* and the *stratum corneum* (18, 19). Consisting of cornified keratinocytes, embedded in a lipid-rich matrix, the 15- to 20- μ m thick *stratum corneum* functions as important physical barrier excluding molecules bigger than 500 Da (20). Interdispersed between keratinocytes are pigment-producing melanocytes and antigen-presenting LCs (19). Through network formation with their dendrites, LCs cover up to 20% of the skin surface

(21), although they only account for 3–5% of the epidermal cells (18). In contrast to the epidermis, the dermis harbours a great diversity of cell types ranging from fibroblasts to macrophages, mast cells, different subsets of dermal dendritic cells (DCs) (22) as well as T cells (19). Moreover, a dense network of lymphatic vessels and blood vessels form the connection to the draining lymph nodes and the systemic circulation (19).

Immunological functions of the skin

As the primary interface between body and environment, the skin not only exerts physical barrier function but also important immune-surveillance function (23). Keratinocytes, LCs, dermal DCs and subsets of T cells together with the local draining lymph nodes form the so-called 'skin-associated lymphoid tissue' (SALT) – a concept formulated by Streilein (24) who was the first to perceive the skin as a 'quasi immunological organ'.

As DCs are key players in tailoring and polarizing the adaptive immune responses (25), understanding the different DC subsets populating the skin (22) is essential. Simplified, skin DCs can be grouped into epidermal LCs and dermal DCs. Generally, LCs are preferentially involved in shaping of the cellular immune response, whereas dermal DCs are more important for regulating B-cell responses (26, 27). Accordingly, LCs preferentially localize within T-cell zones of secondary lymphoid organs, whereas dermal DC preferentially accumulate in proximity to B-cell areas (28). Also, LCs have been demonstrated to efficiently cross-present antigen and to prime CD8⁺ T cells, whereas dermal DCs are required for B-cell isotype switching and induction of IgA. With regard to Th-cell polarization, LCs promote secretion of IL-10 and IL-4 and preferentially elicit Th2-type responses. Activation of dermal DCs on the other hand induces pro-inflammatory cytokines and Th1-type responses (26, 27). Even though this functional dichotomy of different skin DC subsets and their differential activation might explain the wide range of immunological responses obtained after epicutaneous vaccination (29), there is increasing evidence that DCs are not the only cells responsible for shaping adaptive immune responses upon antigen encounter via the skin.

Tissue cells, here keratinocytes, are likely to play a pivotal role in governing adaptive immune responses. The concept of 'the power of the tissue in determining the effector class response' was first introduced by Polly Matzinger (30). Based on the observation that the first trigger for the initiation of an immune response arises in damaged peripheral tissue, she proposed that tissue-derived signals educate resident antigen-presenting cells (APCs) in order to induce a tissue-tailored (and tissue-protective) immune response (30). This concept is supported by recent observations that different types of epithelial cell damage trigger distinct molecular pathways, which promote secretion of specific cytokines shaping the innate and adaptive immune responses (31). Hence, relatively slight stress to the epithelium such as abrasion without penetration has been shown to predominantly induce the secretion

of TSLP, IL-25 and IL-33, which in turn instruct noninflammatory Treg or Th2-type responses. In contrast, as epithelial damage increases, the expression of additional molecules such as IL-1 α , IL-6 and TNF skew the immune response towards a Th1-type response (31).

Proposing that the degree of epithelial damage is the key event determining immune response polarization not only gives consideration to an important role of keratinocytes in shaping adaptive immune responses but also provides an explanation for the observed functional dichotomy of different DC: while superficial damage induces a 'noninflammatory' response transmitted by LCs, deeper epithelial damage induces a 'pro-inflammatory' response that is carried by dermal DC subsets. This concept might not only explain the different types of immune responses observed after epicutaneous immunization but it also opens the possibility to deliberately shape the immune responses by the degree skin barrier disruption prior to epicutaneous antigen administration.

Epicutaneous immunization

Nomenclature

While most of the previous literature refers to the term 'transcutaneous' immunotherapy (TCI) when describing application of a vaccine to the skin, we think that the term 'epicutaneous' is more precise. When other routes of vaccination or immunotherapy are described, it is the site of the application that gives the route its name, such as subcutaneous immunotherapy (SCIT), sublingual immunotherapy (SLIT), intralymphatic immunotherapy (ILIT) intramuscular vaccination (i.m.) or subcutaneous vaccination (s.c.). Following this logic, application 'onto' the skin should be named 'epicutaneous'. Also, the term 'transcutaneous' is misleading as this administration route aims to deliver the vaccine 'into' and not 'across' the skin.

Advantages of epicutaneous immunization

Epicutaneous vaccination targets especially the outermost layer of the skin, the epidermis, which is characterized by three key features: (i) barrier function exerted by keratinocytes; (ii) potent immune surveillance exerted in the first place by keratinocytes and LCs; and (iii) absence of vascularization (19, 23). Taking advantage of the high density of LCs that are sitting in a nonvascularized environment and cover nearly 20% of the skin surface (21), the epicutaneous vaccination route has the potential to be highly efficacious and safe. Accordingly, antigen presentation to the local draining lymph nodes by skin DCs has been shown to efficiently induce systemic IgM and IgG as well as mucosal IgA responses (32). Furthermore, vaccination through the skin has been demonstrated to induce potent cellular CD8⁺ T-cell responses (33). Able to induce such diverse immunological responses, epicutaneous immunization has been tested as treatment for various disorders such as infectious diseases (34, 35), cancer (33), Alzheimer's disease (36), experimental encephalomyelitis (37, 38) and, last but not least, IgE-mediated allergies (39–41).

Challenges to epicutaneous immunization

Although the skin is readily accessible, simple topical application of a vaccine does typically not induce an adequate immune response because of the low permeability of the *stratum corneum* (20). Historically, this physical barrier was disrupted by scratching with a needle, a method called scarification (32). Today, this is replaced by adhesive tape stripping (39) or abrasive methods (42). In the future, these methods might be replaced by the use of microneedle arrays (18, 43). Of note, such epidermal barrier disruption not only increases permeability of the skin but also exerts an immunostimulatory effect through the activation of keratinocytes. Upon stimulation by physical or chemical 'danger signals', keratinocytes have been demonstrated to release pro-inflammatory cytokines which in turn increase antigen uptake and maturation of skin DCs (31, 44). Alternatively, penetration can also be enhanced by skin hydration over a period of at least 4–10 h (45), e.g. by application of an occlusive patch leading to sweat accumulation (41, 46).

Historical view on epicutaneous immunization

The first documented application of epicutaneous vaccination goes back more than 3000 years, when the first immunization against smallpox was practiced in India by administering dry scabs of smallpox lesions onto scarified skin of healthy individuals, a procedure called 'variola' (Fig. 1). This historic form of epicutaneous vaccination substantially reduced mortality of smallpox from 30% during natural outbreaks to <1% (47). Despite the skin's ancient role as immunization organ, the epicutaneous route of immunization has only been re-discovered at the beginning of the last century. Interestingly, 'skin immunization' was then reintroduced to treat allergies (48). However, it was not until the beginning of the 21st century, driven by the increasing interest in novel needle-free vaccination routes (32, 49), when epicutaneous vaccination had its second revival. Today, the furthest developed product is epicutaneous vaccination against *Escherichia coli*-induced traveller's diarrhoea (35). In addition, epicutaneous vaccination has been successfully tested in animal models against infection with helicobacter pylori (50), influenza virus (43) and diphtheria toxin (51). The protective mechanism in all of these applications relies on the induction of humoral immunity dominated by IgG1 and IgA. Recent studies testing epicutaneous vaccination against HIV also found the induction of mucosal cytotoxic T cells together with the secretion of mucosal antibodies (52). Another field of application is cancer immunotherapy. Several groups achieved promising results with epicutaneous immunotherapy against skin cancer based on induction of potent CD8⁺ T-cell responses (33, 53). In contrast to all these applications, where strong immune responses were induced, one group also showed induction of tolerance after epicutaneous vaccination, which was able to inhibit experimental allergic encephalomyelitis (37, 38).

These divergent immune outcomes after epicutaneous vaccination underline the immense potential of the skin as target

Developments in allergen-specific immunotherapy

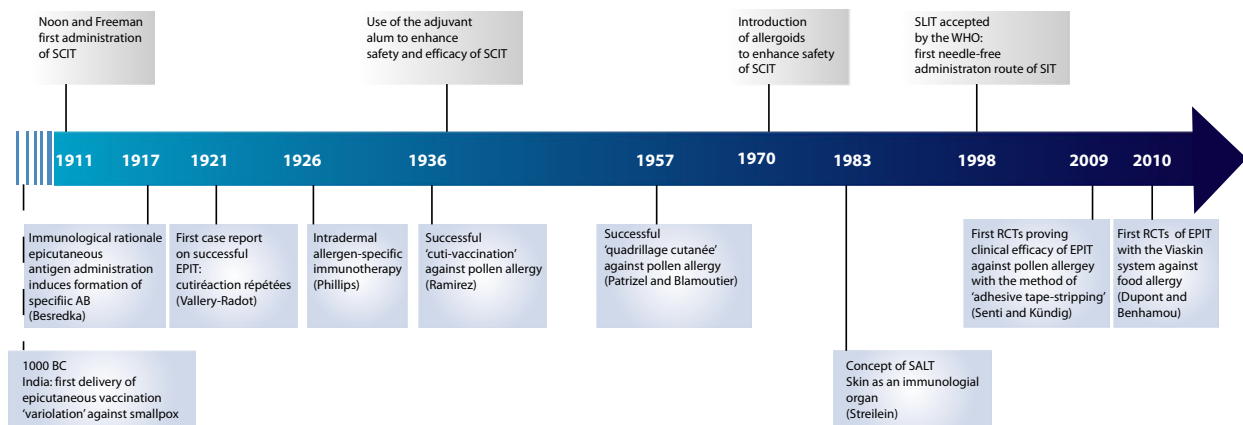


Figure 1 Timeline for the developments in allergen-specific immunotherapy. On the top: Development of currently approved forms

of allergen-specific immunotherapy. On the bottom: Development of epicutaneous allergen-specific immunotherapy.

for various forms of immunotherapy. Here, we specially focus on the role of the skin in allergen-specific immunotherapy.

Epicutaneous allergen-specific immunotherapy in the past (Table 1)

The immunological rationale for use of the epicutaneous administration route of SIT was set in 1917 when Besredka demonstrated that epicutaneous antigen administration was able to induce the formation of specific antibodies (54). Soon after, the first case study on successful EPIT was reported in 1921 (48). Based on the observation that allergen administration on scarified skin, at that time routinely used to verify a patients sensitization, was able to produce systemic allergic symptoms in allergic patients, Vallery-Radot, suggested that such 'cutiréactions répétées' could be able to desensitize a patient. This method was indeed successful in curing an allergic patient from his horse-hair-induced asthma from which he had suffered for 19 years (48).

A decade later, when the risk of suffering a 'pollen shock' was realized to be a considerable danger when administering allergen subcutaneously to highly sensitized patients, a similar method – called intradermal allergen-specific immunotherapy – received attention (55, 56). Based on the observation that hay fever patients occasionally experienced symptom amelioration after 'intradermal pollen tests', E. W. Phillips (56) started to treat very sensitive patients as well as those desiring co-seasonal treatment by the administration of pollen extract 'into the substance of the skin, the same as an intradermal test...'. Strikingly, such intradermal allergen-specific immunotherapy proved to be both safe and highly efficacious leading to symptom relief after administration of three doses only (56). At the same time, M. A. Ramirez obtained similar results while treating grass pollen allergic patients with a method called 'cutivaccination', consisting of the administration of pollen extract on scarified skin (54). Based on these results, it was suggested already in the 1930s that the subcutaneous

route might not be optimal for administration of SIT: '... knowledge of the epidermis as an immunologic organ is still meagre...it may be theoretically possible that a more effective desensitization may be attained by this route than by the subcutaneous one' (55).

Recalling these early successful reports, French allergologists substantially contributed to the revival of EPIT mid of the last century (54, 57, 58). Pautrizel administered the allergen extract onto slightly rubbed epidermis. Even though the reported results were excellent, a large number of applications were necessary until symptom relief was observed (58). Blamoutier, in contrast, applied the allergen drops onto heavily scarified skin (54, 57): 'On the proximal volar aspect of the lower arm, in a square area of 4 × 4 cm, chessboard-like horizontal and vertical scratches are made with a needle... These scratches should be superficial and not cause bleeding' (59).

Each of such epicutaneous treatment applications aimed at producing a wheal-like reaction in the scarified area surrounded by an erythematous halo (54, 57). This method, known as 'quadrillage cutané', was performed co-seasonally conveying rapid symptom relief, which lasted up to several weeks. Therefore, a total of four epicutaneous treatments were sufficient on average to confer symptom relief or considerable symptom amelioration during a whole pollen season (54, 57). Consistently, allergic side-effects were observed only rarely when allergen was applied via the skin and if nevertheless occurring, these reactions were at all the times milder than under conventional SCIT (54, 56–58). These promising results were supported by several studies performed in the subsequent years all over Europe, from Switzerland (59, 60) to Portugal (61). Overall, symptom relief was obtained rapidly and allowed for co-seasonal treatment. The reported treatment success rates of 80% exceeded the success rates under conventional SCIT (59). Despite such successful results with the French '*méthode de quadrillage cutané*' reports on this promising administration route disappeared into oblivion for almost half a century.

Table 1 Historical development of epicutaneous allergen-specific immunotherapy

Disease	No. of subjects	Treatment			Results				References
		Skin pretreatment	Duration	Dose	No. *	Efficacy	Safety	Comments	
Horse-induced asthma	1	Scarification 'cutiréactions répétées'	-3 months -First 2 months daily treatment	Small doses		Relief of asthmatic reaction that was existing for 19 years	Asthmatic crises at treatment start		Vallery-Radot (48)
Pollinosis		Intradermal	-Co-seasonal -Daily application -Relief after 3–4 doses -Treatment repetition every 10 days	Gradually increasing	29	Complete relief or considerable symptom amelioration in all patients	Very safe in highly sensitized patients	Relief was proportionate to the vigour of the local reaction	Phillips (56)
Pollinosis		Slight skin scarification	-Pre- and Co-seasonal -2–3 treatment per week -3 years of treatment for prolonged effect and booster every season	Gradually increasing		100%	No systemic allergic reaction	Better results if: -young patients -short history of allergy -monosensitized	Pautrizel (58)
Pollinosis	65	Scarification 'quadrillage cutanée'	-Co-seasonal -4 treatments per season -Repeated every season	Gradually increasing	34 23 6 2	Fast relief Considerable amelioration Partial amelioration No effect	Generalized grade I and II systemic reactions	Treatment failures mostly if: -previous subcutaneous allergen-specific immunotherapy (SCIT) -polysensitization	Blamoutier (54, 57)
	108	Before treatment: antihistamine			51 36 18 3	Very good results Good results Mediocre No effect	Especially after first application or if rest period of 30 min was not respected		
Pollinosis	75	Scarification 'quadrillage cutanée'	-Co-seasonal -1–4 treatments per season -Repeated every season	Gradually increasing	61 14	Amelioration of symptoms No effect	No systemic allergic reaction	Generally: no long-term effect	DuPan (60)

Table 1 (Continued)

Disease	No. of subjects	Treatment		Duration	Dose	Results			Comments	References
		Skin pretreatment				No. *	Efficacy	Safety		
Pollinosis	42	Scarification 'quadrillage cutané'		-Co-seasonal 6-12 treatments every season on average	Gradually increasing	35	Considerable improvement	Side-effects very rarely	Treatment was more successful than conventional SCIT	Eichenberger (59)
	72					7	Little effect			
	60			-Generally symptom relief within 24 h		57	Considerable improvement			
						15	Little effect			
						52	Considerable improvement			
						8	Little effect			
	141					118	Considerable improvement			
						23	Little effect			
Pollinosis	27	Scarification 'quadrillage cutané'		-Co-seasonal		10	Very good results		Treatment is less successful if:	Palma (61)
						11	Mediocre		-Polysensitization	
						3	No effect		-Allergic asthma	
									-Previous SCIT	

*Number of patients.

Epicutaneous allergen-specific immunotherapy in the 21st century (Table 2)

EPIT with skin barrier disruption: method of adhesive tape stripping

In the context of increasing interest in needle-free vaccine administration routes (32, 49) and encouraged by promising results reported by Glenn (34) who demonstrated successful induction of humoral immune responses after 'transcutaneous' vaccine delivery, the historical observations on successful EPIT returned to mind.

Driven by the idea to find a patient-convenient application route of SIT in order to increase its attractiveness and based on the good accessibility of the skin and its high density of potent immune cells, our group performed three clinical trials to test efficacy and safety of EPIT. In order to keep epithelial barrier disruption minimal, we replaced skin scarification by the adhesive tape stripping method (39). Besides enhancing penetration of the allergens through removal of the *stratum corneum* (62), repeated tape stripping also functions as a 'physical' adjuvant through activation of keratinocytes, which then secrete various pro-inflammatory cytokines (IL-1, IL-6, IL-8, TNF- α and INF- γ) that favour maturation and emigration of DCs to the draining lymph nodes (63, 64). Results from the first pilot trial (NCT00457444) revealed that patients treated with a total of 12 pollen extract containing patches experienced significant alleviation of hay fever symptoms compared to placebo-treated patients. In line with the above-described 'historical' study results, no severe systemic allergic reactions were reported. The only adverse events observed were very mild local eczematous reactions under the skin patch in a minority of patients (39). Encouraged by these results, a second phase I/IIa trial including a total of 132 grass pollen allergic patients was initiated to find the optimal treatment dose of EPIT. Enrolled patients were treated co-seasonally with a total of six patches (Senti et al. manuscript in preparation, NCT00719511). A third clinical trial has been started to investigate the immunological changes induced during EPIT (NCT00777374). Our results were meanwhile confirmed by an independent group that demonstrated efficacy and safety of EPIT in grass pollen allergic children. Hay fever symptoms as well as the use of antihistamines were significantly reduced in the active treatment group (40).

EPIT using hydration to enhance permeability

In contrast to the original method of 'quadrillage cutané' (54, 57) and in contrast to the 'method of adhesive tape stripping' (39), both aiming at disrupting the skin barrier prior to allergen administration, a French group recently developed an alternative form of EPIT based on allergen delivery to the intact skin using an occlusive epidermal delivery system (Viaskin® EDS) (41, 46, 65). Initially developed for diagnostic purposes as an alternative system to the conventional Finn chamber used in atopy patch test (66), Viaskin® relies on the ability to deliver whole protein molecules to the skin (46, 65). Perspiration generated under an occlusive chamber not only dissolves the lyophilized allergen protein that is loaded on the

Table 2 Epicutaneous allergen-specific immunotherapy in the 21st century

Disease	Design	Number of subjects	Treatment		Duration	Dose	Results		
			Skin	Pretreatment			Efficacy	Safety	Immunological effects
Pollinosis (grass pollen)	Phase I	37		Tape stripping	-Pre- and Co-seasonal	-Unchanged during treatment	-Clinically and statistically significant 70% improvement of hay fever symptoms	Local erythema and eczema	Eczema at patch application site
	Double-blind RCT			Patch	-12 patches (48 h in place)	-1.5 x atopy patch test dose	-Trend towards increased allergen tolerance in the nasal provocation test	No systemic allergic reactions	indicates T cell activation
Pollinosis (grass pollen)	Phase I/IIa	132		Tape stripping	-Pre- and Co-seasonal	-Unchanged during treatment			No immunological parameters studied
	Double-blind RCT			Patch application	-1 treatment season	-3 treatment dose-arms			
Pollinosis (grass pollen)	Phase I/IIa	98		Tape stripping	-Pre- and Co-seasonal	-Unchanged during treatment		No systemic or local allergic reactions	
	Double-blind RCT			Patch	-1 treatment season				
Pollinosis (grass pollen)	Phase I	15		? pre-treatment	-Pre- and Co-seasonal	-Unchanged during treatment	-Significant reduction in symptoms		No immunological parameters studied
	Double-blind RCT			Patch	-12 patches (24 h in place) treatment		-Significant reduction in antihistamine dose		
Food allergy (cow milk)	Phase I	19 (children)		Intact skin	-observation during 1 year)		-No significant change in prick test		
	Double-blind RCT			Viaskin epidermal delivery system (EDS)	-3 EDS applications per week (48 h in place) for 3 months			Local erythema and eczema	
Food allergy (peanut allergy)	Phase Ib	110 (children)		Intact skin		-Different treatment dose-arms		No anaphylaxis	
	Double-blind RCT			Viaskin EDS		-Different administration time arms			
Food allergy (peanut allergy)	Phase II	52 (children)		Intact skin					
	Double-blind RCT			Viaskin EDS					

Viaskin[®] EDS (46, 65) but also hydrates the cornified layers of the stratum corneum thereby enhancing its penetration. Delivered via such EDS, protein has been demonstrated to accumulate in the stratum corneum, where it efficiently targets immune cells of the superficial skin layer (67), that rapidly migrate to the draining lymph nodes (46). In murine studies, EPIT using the Viaskin[®] EDS has proven equivalent efficacy as SCIT in preventing allergic airway reactions upon inhalative allergen challenge (46). Furthermore, EPIT harnessing the properties of this occlusive chamber proved to be an efficacious treatment for food allergy as measured by prevention of mast cell degranulation upon oral allergen challenge in mice (65). A clinical pilot trial lanced to test clinical efficacy and safety of EPIT using the Viaskin[®] EDS in children suffering from cow's milk allergy showed a tendency towards an increased cumulative tolerance dose after a 3-month treatment period, but missed statistical significance. Treatment was well tolerated with no systemic anaphylactic reactions; however, a significant increase in local eczematous skin reactions was observed (41). Such good safety results are crucial especially when considering the use of EPIT as treatment option for food allergies, for which conventional SCIT is impractical because of an unacceptably high rate of anaphylactic reactions (68). To substantiate these early findings and aiming to develop a definitive therapeutic option for food allergic patients, a phase I (NCT01170286) and a phase II trial (NCT01197053) have recently been initiated to test treatment efficacy of EPIT with the Viaskin[®] EDS in peanut allergic patients.

The current practice of EPIT: a comparison between the two methods

Clinically, both methods of EPIT, either with or without epidermal barrier disruption, were accompanied by the amelioration of allergic symptoms (39, 41). Remarkably, however, EPIT after skin disruption either by 'scarification' or 'adhesive tape stripping' induced rapid symptom amelioration after administration of a few treatments only (39, 54, 57, 59). In contrast, EPIT using the Viaskin[®] EDS was not able to demonstrate a significant treatment effect after a 3-month treatment period, although a trend towards improvement was observed. The authors speculated that a longer treatment period might increase the treatment effect (41). Such reasoning might indeed be true, as it was observed early in the development of EPIT, that Pautrizel (58), who applied the allergen onto slightly scratched skin only, needed to treat his patients substantially longer than Blamoutier, who applied the allergen onto heavily scarified skin (57).

Unfortunately, the immunological changes induced by EPIT are only poorly investigated. However, there is increasing evidence, that the way how epicutaneous immunization is carried out determines the immune outcome, inducing either active immunity or tolerance (29). In the light of the current evidence that the degree of skin barrier disruption plays an essential role in determining immune response polarization (31), the immunological changes induced after EPIT using the method of adhesive tape stripping are likely to be different from those observed after by EPIT with the Viaskin[®]

EDS. Hence, a heavily disrupted skin barrier has been observed to polarize the immune response towards Th1, whereas slight skin barrier disruption rather induces a non-inflammatory Th2/Treg-dominated response (Fig. 2) (31). Clinical studies focusing on the immunological changes induced after both methods of EPIT might help to rationally assess the advantages and limitations of each one. Fundamental to the successful use of EPIT as novel administration route for SIT is the absence of life-threatening systemic allergic side-effects that was observed with both methods (39, 41). This matter of fact is an indispensable requirement for its promotion as a self-administrable treatment option for IgE-mediated allergies.

Future directions

Even though EPIT has proven its efficacy in animal and in human studies, there still is potential to enhance its clinical efficacy and to reduce treatment duration and the number of patch applications (69). A promising strategy to achieve this objective is to deliver the allergen extract together with an adjuvant, a rational step that mirrors the development of SCIT for which efficacy was considerably enhanced by adding the adjuvant Alum to the allergy vaccine (13, 70). Alum, however, today still the adjuvant used in the majority of marketed vaccines (10), is not suitable for epicutaneous administration (71). Thus far, cholera toxin and heat-labile enterotoxin (LT) have been successfully used as adjuvants in epicutaneous vaccination against infectious diseases of mice and humans (35, 71, 72). On the other hand, imidazoquinolines and CpG are currently tested as adjuvants for epicutaneous vaccination against cancer (29, 53). Yet, none of these adjuvants seems appropriate for use in SIT – a context that ideally requires immune-modulation towards Th1 or Treg, while inducing potent 'blocking' antibodies (4, 6). Therefore, we recently tested the immune-enhancing and immune-modulatory potential of diphenylcyclopropenone when used as adjuvant in EPIT (von Moos et al., manuscript in preparation). Precise targeting of the skins' APC's with microneedle arrays using suitable needle length might be another approach to increase treatment efficacy. Although initially designed for drug delivery purposes, microneedle arrays are more and more frequently used in epicutaneous immunization studies (18). Recently, a single vaccination using a dissolvable polymer microneedle patch has been demonstrated to induce protective immune responses against influenza virus infection in mice (43). This novel technology might therefore bear the potential to design the ideal vaccine for desensitization conferring protection after a single administration. Last but not least, encapsulation of allergen into nanoparticles or liposomes might be an additional strategy to enhance treatment efficacy (18).

Outlook

In the light of the increasing prevalence of allergic disease (2, 73), which strongly contrasts the low percentage of patients choosing to undergo SCIT (8, 9), research during the

Epicutaneous immunization

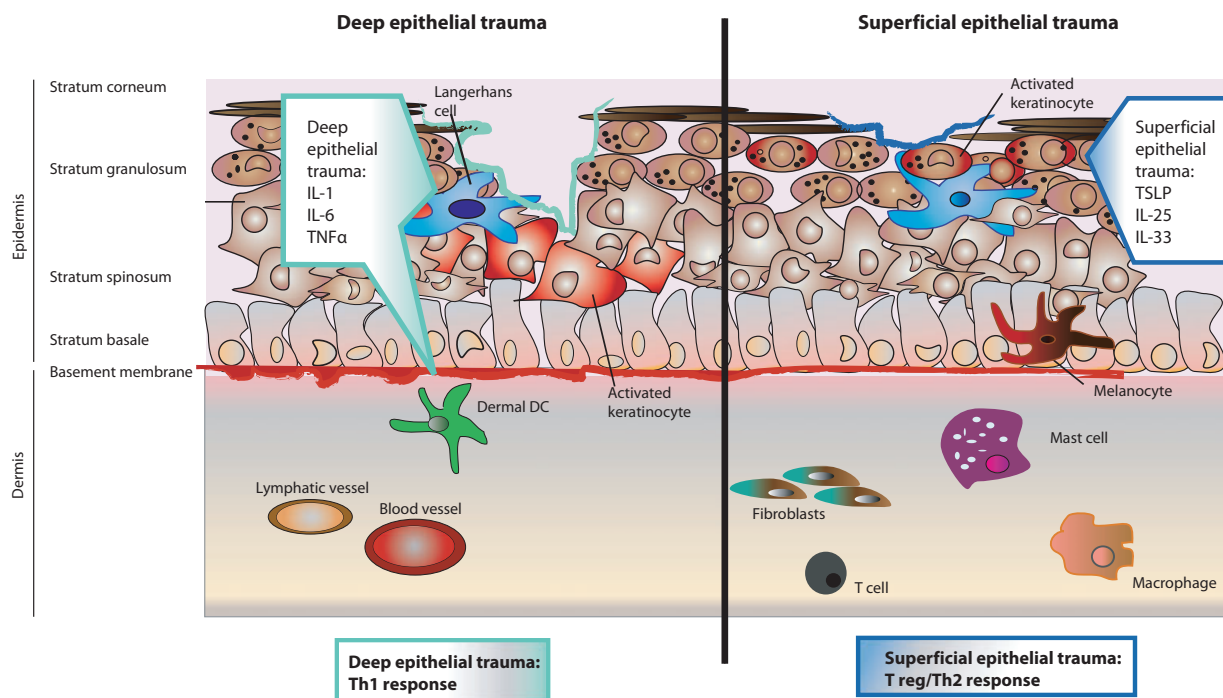


Figure 2 Potential mechanism of epicutaneous immunization. Antigen administration on heavily disrupted skin barrier induces the release of IL-1, IL-6 and TNF α by activated keratinocytes, which 'imprint' tissue-resident DCs (LCs and dermal DCs) to induce a Th1-type response in the draining lymphnode (on the

left). On the other hand, antigen administration on only slightly disrupted skin barrier induces the release of TSLP, IL-25 and IL-33 by activated keratinocytes, leading to activation of LC which in turn induce a Treg/Th2-type response in the draining lymph node (on the right).

next century should aim at optimization of current SIT methods in order to increase its attractiveness. Optimization of allergen immunotherapy should (i) deliver allergen via a route that efficiently targets professional APCs, (ii) use optimal adjuvants, (iii) avoid allergen delivery to highly vascularized sites as to minimize systemic allergic side-effects and (iv) be convenient for the patient, i.e. self-administrable and painless. Epicutaneous allergen-specific immunotherapy holds promise in all four aspects: (i) the epidermis contains a high number of potent APCs, (ii) adjuvants can be topically administered and/or physical or chemical trauma to keratinocytes may already act as a 'optimal physical adjuvant', (iii) the epidermis is nonvascularized and (iv) epicutaneous administration can be done at home and is painless.

Many allergologists – including ourselves when we started our project – are not aware that EPIT looks back on a long history. First reports date back up to 90 years (48), yet this route of administration for SIT has only attracted attention in recent years. Correspondingly, the understanding of the immunological processes occurring in the skin is only slowly growing and the distinct role of diverse skin DC subsets and epithelial cytokines is far from being disentangled. Nevertheless, there is increasing evidence that keratinocytes not only exert a shear physical barrier function but also actively polarize the immune response via secretion of epithelial cytokines.

This concept was first mentioned by Polly Matzinger (30, 74) and only recently renewed by Mahima Swamy (31) who defined the term 'epimmunome' to describe molecules used by epithelial cells to instruct immune cells. The potential of the skin to induce a variety of different immune responses, dependent on skin preparation prior to antigen administration as well as dependent on the use of different adjuvants, has encouraged the testing of epicutaneous immunization for diverse indications (29). Clinical trials have recently demonstrated the potential of EPIT to ameliorate allergic rhinoconjunctivitis (39) as well as food allergy (41). Yet, it still needs to be elucidated whether the clinically observed effect is mediated by blocking antibodies, upregulation of a Th1 response or induction of Treg cells. Advances in the understanding of these mechanisms together with the adept use of epicutaneously active adjuvants or microneedle arrays are likely to considerably increase efficacy of EPIT in the near future.

The two outstanding characteristics of EPIT consist in its favourable safety profile and its needle-free administration mode enabling self-administration. These two features might also allow its application in two 'niche situations': treatment of food allergy and promotion of SIT in children. Until today, there is no definite therapeutic option to treat food allergy, as conventional SCIT is associated with an unacceptably high risk of anaphylactic side-effects. Dietary aller-

gen restriction and immediate application of self-injectable epinephrine is therefore the current standard of care (68). By reason of its outstanding safety profile, because of restricted allergen access to the vascular system and allergen accumulation in the nonvascularized *stratum corneum*, EPIT has the potential to revolutionize therapeutic options for food allergies, as demonstrated in a first clinical trial (41). Besides food allergic patients, children may represent another particularly interesting target population for EPIT.

While afraid of needles, it is especially children who benefit most from SIT (75), as its administration early in the course of allergic diseases has the potential to stop disease progression to asthma, which represents a considerable health burden. Such reasoning might highlight in the development of a preventive needle-free, patch-based allergy vaccine, accepted as a part of the WHO recommended 'early childhood' vaccination programme, to conquer the epidemic of the 21st century.

References

- Bostock J. Case of the periodical affection of the eyes and chest. *Med-Chir Trans* 1819; **10**:161–165.
- Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK et al. World-wide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 2006; **368**:733–743.
- Holgate ST, Polosa R. Treatment strategies for allergy and asthma. *Nat Rev Immunol* 2008; **8**:218–230.
- Akdis M, Akdis CA. Therapeutic manipulation of immune tolerance in allergic disease. *Nat Rev Drug Discov* 2009; **8**:645–660.
- Noon L. Prophylactic inoculation against hay fever. *Lancet* 1911; **177**:1572–1573.
- Larche M, Akdis CA, Valenta R. Immunological mechanisms of allergen-specific immunotherapy. *Nat Rev Immunol* 2006; **6**:761–771.
- Frew AJ. Allergen immunotherapy. *J Allergy Clin Immunol* 2010; **125**(2 Suppl. 2):S306–S313.
- Cox L, Calderon MA. Subcutaneous specific immunotherapy for seasonal allergic rhinitis: a review of treatment practices in the US and Europe. *Curr Med Res Opin* 2010; **26**:2723–2733.
- Cox LS, Larenas Linnemann D, Nolte H, Weldon D, Finegold I, Nelson HS. Sublingual immunotherapy: a comprehensive review. *J Allergy Clin Immunol* 2006; **117**:1021–1035.
- Marrack P, McKee AS, Munks MW. Towards an understanding of the adjuvant action of aluminium. *Nat Rev Immunol* 2009; **9**:287–293.
- Drachenberg KJ, Wheeler AW, Stuebner P, Horak F. A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. *Allergy* 2001; **56**:498–505.
- Tulic MK, Fiset PO, Christodoulouopoulos P, Vaillancourt P, Desrosiers M, Lavigne F et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. *J Allergy Clin Immunol* 2004; **113**:235–241.
- Focke M, Swoboda I, Marth K, Valenta R. Developments in allergen-specific immunotherapy: from allergen extracts to allergy vaccines bypassing allergen-specific immunoglobulin E and T cell reactivity. *Clin Exp Allergy* 2010; **40**:385–397.
- Mutschlechner S, Deifl S, Bohle B. Genetic allergen modification in the development of novel approaches to specific immunotherapy. *Clin Exp Allergy* 2009; **39**:1635–1642.
- Canonica GW, Bousquet J, Casale T, Lockett RF, Baena-Cagnani CE, Pawankar R et al. Sub-lingual immunotherapy: World Allergy Organization Position Paper 2009. *Allergy* 2009; **64**(Suppl. 91):1–59.
- Senti G, Prinz Vavricka BM, Erdmann I, Diaz MI, Markus R, McCormack SJ et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. *Proc Natl Acad Sci USA* 2008; **105**:17908–17912.
- Senti G, Johansen P, Kundig TM. Intralymphatic immunotherapy. *Curr Opin Allergy Clin Immunol* 2009; **9**:537–543.
- Bal SM, Ding Z, van Riet E, Jiskoot W, Bouwstra JA. Advances in transcutaneous vaccine delivery: do all ways lead to Rome? *J Control Release* 2010; **148**:266–282.
- Nestle FO, Di Meglio P, Qin JZ, Nickoloff BJ. Skin immune sentinels in health and disease. *Nat Rev Immunol* 2009; **9**:679–691.
- Bos JD, Meinardi MM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. *Exp Dermatol* 2000; **9**:165–169.
- Babiuk S, Baca-Estrada M, Babiuk LA, Ewen C, Foldvari M. Cutaneous vaccination: the skin as an immunologically active tissue and the challenge of antigen delivery. *J Control Release* 2000; **66**:199–214.
- Merad M, Ginhoux F, Collin M. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. *Nat Rev Immunol* 2008; **8**:935–947.
- Kupper TS, Fuhlbrigge RC. Immune surveillance in the skin: mechanisms and clinical consequences. *Nat Rev Immunol* 2004; **4**:211–222.
- Streilein JW. Skin-associated lymphoid tissues (SALT): origins and functions. *J Invest Dermatol* 1983; **80**(Suppl.):12s–16s.
- Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat Rev Immunol* 2003; **3**:984–993.
- Klechevsky E, Morita R, Liu M, Cao Y, Coquery S, Thompson-Snipes L et al. Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. *Immunity* 2008; **29**:497–510.
- Ueno H, Schmitt N, Klechevsky E, Pedroza-Gonzalez A, Matsui T, Zurawski G et al. Harnessing human dendritic cell subsets for medicine. *Immunol Rev* 2010; **234**:199–212.
- Kissenpfennig A, Henri S, Dubois B, Laplace-Builhe C, Perrin P, Romani N et al. Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. *Immunity* 2005; **22**:643–654.
- Stoitzner P, Sparber F, Tripp CH. Langerhans cells as targets for immunotherapy against skin cancer. *Immunol Cell Biol* 2010; **88**:431–437.
- Matzinger P. Friendly and dangerous signals: is the tissue in control? *Nat Immunol* 2007; **8**:11–13.
- Swamy M, Jamora C, Havran W, Hayday A. Epithelial decision makers: in search of the 'epimimmune'. *Nat Immunol* 2010; **11**:656–665.
- Mitragotri S. Immunization without needles. *Nat Rev Immunol* 2005; **5**:905–916.
- Yagi H, Hashizume H, Horibe T, Yoshinari Y, Hata M, Ohshima A et al. Induction of therapeutically relevant cytotoxic T lymphocytes in humans by percutaneous peptide immunization. *Cancer Res* 2006; **66**:10136–10144.
- Glenn GM, Taylor DN, Li X, Frankel S, Montemarano A, Alving CR. Transcutaneous immunization: a human vaccine delivery strategy using a patch. *Nat Med* 2000; **6**:1403–1406.

35. Frech SA, Dupont HL, Bourgeois AL, McKenzie R, Belkind-Gerson J, Figueroa JF et al. Use of a patch containing heat-labile toxin from *Escherichia coli* against travellers' diarrhoea: a phase II, randomised, double-blind, placebo-controlled field trial. *Lancet* 2008;**371**:2019–2025.
36. Nikolic WV, Bai Y, Obregon D, Hou H, Mori T, Zeng J et al. Transcutaneous beta-amyloid immunization reduces cerebral beta-amyloid deposits without T cell infiltration and microhemorrhage. *Proc Natl Acad Sci USA* 2007;**104**:2507–2512.
37. Bynoe MS, Viret C. Antigen-induced suppressor T cells from the skin point of view: suppressor T cells induced through epicutaneous immunization. *J Neuroimmunol* 2005;**167**:4–12.
38. Bynoe MS, Evans JT, Viret C, Janeway CA Jr. Epicutaneous immunization with autoantigenic peptides induces T suppressor cells that prevent experimental allergic encephalomyelitis. *Immunity* 2003;**19**:317–328.
39. Senti G, Graf N, Haug S, Ruedi N, von Moos S, Sonderegger T et al. Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. *J Allergy Clin Immunol* 2009;**124**:997–1002.
40. Agostinis F, Forti S, Di Berardino F. Grass transcutaneous immunotherapy in children with seasonal rhinoconjunctivitis. *Allergy* 2010;**65**:410–411.
41. Dupont C, Kalach N, Soulaïnes P, Legoue-Morillon S, Piloquet H, Benhamou PH. Cow's milk epicutaneous immunotherapy in children: a pilot trial of safety, acceptability, and impact on allergic reactivity. *J Allergy Clin Immunol* 2010;**125**:1165–1167.
42. Frerichs DM, Ellingsworth LR, Frech SA, Flyer DC, Villar CP, Yu J et al. Controlled, single-step, stratum corneum disruption as a pretreatment for immunization via a patch. *Vaccine* 2008;**26**:2782–2787.
43. Sullivan SP, Koutsouanos DG, Del Pilar Martin M, Lee JW, Zarnitsyn V, Choi SO et al. Dissolving polymer microneedle patches for influenza vaccination. *Nat Med* 2010;**16**:915–920.
44. Wood LC, Jackson SM, Elias PM, Grunfeld C, Feingold KR. Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice. *J Clin Invest* 1992;**90**:482–487.
45. Tan G, Xu P, Lawson LB, He J, Freytag LC, Clements JD et al. Hydration effects on skin microstructure as probed by high-resolution cryo-scanning electron microscopy and mechanistic implications to enhanced transcutaneous delivery of biomacromolecules. *J Pharm Sci* 2010;**99**:730–740.
46. Mondoulet L, Dioszeghy V, Ligouis M, Dhelft V, Dupont C, Benhamou PH. Epicutaneous immunotherapy on intact skin using a new delivery system in a murine model of allergy. *Clin Exp Allergy* 2010;**40**:659–667.
47. Stewart AJ, Devlin PM. The history of the smallpox vaccine. *J Infect* 2006;**52**:329–334.
48. Vallery-Radot P, Hangenau J. Asthme d'origine équine. Essai de désensibilisation par des cutiréactions répétées. *Bull Soc Méd Hôp Paris* 1921;**45**:1251–1260.
49. Jodar L, Duclos P, Miltien JB, Griffiths E, Aguado MT, Clements CJ. Ensuring vaccine safety in immunization programmes – a WHO perspective. *Vaccine* 2001;**19**:1594–1605.
50. Hickey DK, Aldwell FE, Tan ZY, Bao S, Beagley KW. Transcutaneous immunization with novel lipid-based adjuvants induces protection against gastric *Helicobacter pylori* infection. *Vaccine* 2009;**27**:6983–6990.
51. Ding Z, Verbaan FJ, Bivas-Benita M, Bungener L, Huckriede A, van den Berg DJ et al. Microneedle arrays for the transcutaneous immunization of diphtheria and influenza in BALB/c mice. *J Control Release* 2009;**136**:71–78.
52. Belyakov IM, Hammond SA, Ahlers JD, Glenn GM, Berzofsky JA. Transcutaneous immunization induces mucosal CTLs and protective immunity by migration of primed skin dendritic cells. *J Clin Invest* 2004;**113**:998–1007.
53. Rechtsteiner G, Warger T, Osterloh P, Schild H, Radsak MP. Cutting edge: priming of CTL by transcutaneous peptide immunization with imiquimod. *J Immunol* 2005;**174**:2476–2480.
54. Blamoutier P, Blamoutier J, Guibert L. Traitement co-saisonnier de la pollinose par l'application d'extraits de pollens sur des quadrillages cutanés: Résultats obtenus en 1959 et 1960. *Revue Française d'Allergie* 1961;**1**:112–120.
55. Hurwitz SH. Medicine: seasonal hay fever – some problems in treatment. *Cal West Med* 1930;**33**:520–521.
56. Phillips EW. Relief of hay-fever by intradermal injections of pollen extract. *J Am Med Assoc* 1926;**86**:182–184.
57. Blamoutier P, Blamoutier J, Guibert L. Treatment of pollinosis with pollen extracts by the method of cutaneous quadrille ruling. *Presse Med* 1959;**67**:2299–2301.
58. Pautrizel R, Cabanieu G, Bricaud H, Broustet P. Allergenic group specificity & therapeutic consequences in asthma; specific desensitization method by epicutaneous route. *Sem Hop* 1957;**33**:1394–1403.
59. Eichenberger H, Storck H. Co-seasonal desensitization of pollinosis with the scarification-method of Blamoutier. *Acta Allergol* 1966;**21**:261–267.
60. Martin-DuPan RBF, Neyroud M. Treatment of pollen allergy using the cutaneous checker square method of Blamoutier and Guibert. *Schweiz Rundsch Med Prax* 1971;**60**:1469–1472.
61. Palma-Carlos AG. Traitement co-saisonnier des pollinoses au Portugal par la méthode des quadrillages cutanés. *Revue Française d'Allergie* 1967;**7**:92–95.
62. Dickel H, Goulioumis A, Gambichler T, Fluhr JW, Kamphowe J, Altmeyer P et al. Standardized tape stripping: a practical and reproducible protocol to uniformly reduce the stratum corneum. *Skin Pharmacol Physiol* 2010;**23**:259–265.
63. Nickoloff BJ, Naidu Y. Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin. *J Am Acad Dermatol* 1994;**30**:535–546.
64. Dickel H, Gambichler T, Kamphowe J, Altmeyer P, Skrygan M. Standardized tape stripping prior to patch testing induces upregulation of Hsp90, Hsp70, IL-33, TNF-alpha and IL-8/CXCL8 mRNA: new insights into the involvement of 'alarmins'. *Contact Dermatitis* 2010;**63**:215–222.
65. Mondoulet L, Dioszeghy V, Vanoirbeek JA, Nemery B, Dupont C, Benhamou PH. Epicutaneous immunotherapy using a new epicutaneous delivery system in mice sensitized to peanuts. *Int Arch Allergy Immunol* 2010;**154**:299–309.
66. Kalach N, Soulaïnes P, de Boissieu D, Dupont C. A pilot study of the usefulness and safety of a ready-to-use atopy patch test (Diallertest) versus a comparator (Finn Chamber) during cow's milk allergy in children. *J Allergy Clin Immunol* 2005;**116**:1321–1326.
67. Soury D, Barratt G, Ah-Leung S, Legrand P, Chacun H, Ponchel G. Skin localization of cow's milk proteins delivered by a new ready-to-use atopy patch test. *Pharm Res* 2005;**22**:1530–1536.
68. Scurlock AM, Jones SM. An update on immunotherapy for food allergy. *Curr Opin Allergy Clin Immunol* 2010;**10**:587–593.
69. Werfel T. Epicutaneous allergen administration: a novel approach for allergen-specific immunotherapy? *J Allergy Clin Immunol* 2009;**124**:1003–1004.
70. Harris MC. HAY FEVER-a comparative clinical evaluation of treatment with aqueous pollen extracts, alum-precipitated pyridine pollen extracts and aqueous pollen in oil emulsions. *Calif Med* 1962;**97**:286–290.
71. Scharton-Kersten T, Yu J, Vassell R, O'Hagan D, Alving CR, Glenn GM. Transcutaneous immunization with bacterial ADP-ribosylating exotoxins, subunits, and unrelated adjuvants. *Infect Immun* 2000;**68**:5306–5313.

72. Glenn GM, Rao M, Matyas GR, Alving CR. Skin immunization made possible by cholera toxin. *Nature* 1998;**391**:851.
73. Devereux G. The increase in the prevalence of asthma and allergy: food for thought. *Nat Rev Immunol* 2006;**6**:869–874.
74. Matzinger P. The danger model: a renewed sense of self. *Science* 2002;**296**:301–305.
75. Hankin CS, Cox L, Lang D, Bronstone A, Fass P, Leatherman B et al. Allergen immunotherapy and health care cost benefits for children with allergic rhinitis: a large-scale, retrospective, matched cohort study. *Ann Allergy Asthma Immunol* 2010;**104**:79–85.