

Epicutaneous/transcutaneous allergen-specific immunotherapy: rationale and clinical trials

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Purpose of review

IgE-mediated allergies, such as allergic rhinoconjunctivitis and asthma, have become highly prevalent, today affecting up to 35% of the population in industrialized countries. Allergen immunotherapy (also called hyposensitization therapy, desensitization or allergen-specific immunotherapy), the administration of gradually increasing amounts of an allergen, either subcutaneously or via the sublingual or oral route is effective. However, only few allergy patients (<5%) choose immunotherapy, as treatment duration is over years and because allergen administrations are associated with local and in some cases even systemic allergic side effects due to allergen accidentally reaching the circulation. Therefore, ideally the allergen should be administered to a site that contains high numbers of potent antigen-presenting cells in order to enhance efficacy and shorten treatment duration, and ideally that site should also be nonvascularized in order to prevent both systemic distribution of the allergen and systemic allergic side effects. The epidermis, a nonvascularized multilayer epithelium that contains high numbers of potent antigen-presenting Langerhans cells, could therefore be an interesting administration route.

Recent findings

We have recently reintroduced transcutaneous or epicutaneous allergen-specific immunotherapy (EPIT) as treatment option for IgE-mediated allergies. This method was found efficacious and safe. Few applications of allergens using skin patches with a treatment duration of a few weeks were sufficient to achieve lasting relief.

Summary

This review gives an overview on the history, the rationale, and the mechanisms of transcutaneous/epicutaneous immunotherapy.

Keywords

allergy, desensitization, epicutaneous immunotherapy, transcutaneous immunotherapy

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Introduction

IgE-mediated allergies, such as allergic rhinoconjunctivitis and asthma, have become highly prevalent, affecting up to 35% of the population in industrialized countries [1,2]. Allergic rhinitis represents a clinically significant health problem. It is responsible for US\$6 billion annually in healthcare costs in the United States, and frequently causes absence or poor performance at work and at school [3]. Also, allergic rhinoconjunctivitis can evolve into asthma [4].

Rhinoconjunctivitis caused by grass pollen is the most frequent type of IgE-mediated allergy, and grass pollen exposure cannot be avoided by the patient. This is in contrast to house dust mite allergy and cat dander allergy, where the primary recommendation is to avoid exposure to these allergens either by sanitizing the house or giving away the cat. The diagnosis of, for example, grass pollen

allergy is based on history, skin tests, and determination of specific IgE antibodies in the serum.

State of the art

Allergen immunotherapy (also termed hyposensitization therapy, immunologic desensitization, or allergen-specific immunotherapy), usually by subcutaneous administration of gradually increasing amounts of an allergen extract, is an effective treatment for patients with allergic rhinitis, conjunctivitis, and allergy to insect venoms [5–7], and confers a long-term benefit [3]. Subcutaneous immunotherapy (SCIT) also interrupts the so-called ‘atopic march’, the progression of allergic sensitization from a single to multiple allergens [8] and from allergic rhinitis to asthma [9].

Despite these advantages of immunotherapy over symptomatic treatment, only less than 5% of allergy patients

choose immunotherapy [10], mainly because it involves 30–80 doctor visits over 3–5 years. Subcutaneous immunotherapy also requires close supervision by a physician trained in appropriate emergency treatment [6], as it is associated with frequent allergic side effects including life-threatening anaphylaxis [11].

The immunological mechanisms responsible for ameliorating allergy symptoms are still a matter of debate [12,13[•]]. Allergen-specific immunotherapy modulates the allergen-specific immune response, shifting the balance of CD4 T lymphocytes from Th2 towards Th1 with increased interferon-gamma (IFN- γ) and decreased interleukin-4 (IL-4) and interleukin-5 (IL-5) production [14,15]. The production of interleukin-10 (IL-10) by allergen-specific CD4⁺CD25⁺ regulatory T cells [14,15], as well as allergen-specific IgG antibodies, especially IgG4 [16], is increased, but it remains unclear which effector mechanism is ultimately responsible for symptom amelioration [17].

Epicutaneous/transcutaneous vaccination

The skin provides an attractive immune environment for vaccines [18]. Transcutaneous immunization (TCI), the application of vaccines on the skin, induces robust systemic and mucosal antibody responses in animal models and in humans [18,19[•]]. TCI can be performed using either microneedle patches [20] or even needle-free patches to deliver antigens and adjuvants to potent immune cells residing in the skin [21].

In particular, use of the skin as delivery site for vaccines [19[•],22,23] has been spurred by the fact that epidermal dendritic cells, so-called Langerhans cells, are among the most efficient presenters of antigen to the immune system [24,25] and are abundant in the epidermis [26]. Repeated epicutaneous exposure to protein antigens can induce a strong antibody response [27]. After penetration, allergens may also bind to allergen-specific IgE on Langerhans cells [28,29] and may subsequently be presented to T lymphocytes [30,31].

Preparation of the skin

Keratinocytes play an immunologically active role in responding to physical and chemical stimuli that may be associated with epicutaneous or transcutaneous antigen application, such as microneedles or removal of the stratum corneum by abrasives [32] or tape stripping [33,34]. Keratinocytes then produce cytokines, including IL-1, IL-6, IL-8, and TNF- α [35–38]. It is also reported that tape stripping can induce production of IL-12 and IFN- γ in the skin [39] and increase expression of MHC class II, CD86, CD40, CD54, and CD11c on Langerhans cells [34], as well as enhanced expression of Toll-like receptor 9 in keratinocytes [40], producing an environment favoring induction of allergy-protective immune

responses mediated by Langerhans cells and their migration into lymph nodes [35,41,42]. Thus, keratinocytes are active and dynamic participants in the instigation and perpetuation of inflammatory reactions [43].

Tape stripping and abrasive methods to reduce the cornified layer of the epidermis are not only important to activate keratinocytes, but also to enhance penetration of epicutaneously applied substances into the epidermis. Also, with application of a patch alone and subsequent hydration of the cornified layers either by the patch or by accumulation of sweat enhance penetration, especially when using large molecules such as proteins, penetration becomes a critical issue. However, Kenney *et al.* [21] showed that a novel vaccine delivery system using an adhesive patch simplified administration and improved tolerability of a subunit anthrax vaccine. Mice and rabbits were vaccinated with recombinant antigens of *Bacillus anthracis* and the heat-labile toxin of *Escherichia coli*. Serologic changes, levels of toxin-neutralizing antibodies, and pulmonary and nodal responses were monitored. A lethal aerosolized *B. anthracis* challenge model was used to demonstrate efficacy in a mouse model. The level of systemic immunity and protection induced by transcutaneous immunization was comparable to that induced by intramuscular vaccination, and peak immunity could be achieved with only two doses [21].

Glenn *et al.* have demonstrated that cholera toxin [44] and other bacterial toxins [45] can be used as transcutaneous adjuvants for protein immunization through the skin. Repeated application of proteins on the skin in this manner was found to result in the generation of serum and mucosal IgG and IgA antibodies capable of mediating protective immunity against mucosal [46] and systemic [45] toxin challenge. Also in mice, Strid *et al.* [47] demonstrated that epicutaneous immunization can convert subsequent and established antigen-specific T helper cell responses from Th1-type to Th2-type responses.

Frech *et al.* [48] could show an improvement of the immune responses to influenza vaccination in the elderly using an immunostimulant patch. Finally, the same group demonstrated clinical efficacy of a patch vaccine against traveller's diarrhea after only two applications [49].

Early examples of epicutaneous/transcutaneous allergen-specific immunotherapy

In 1957, Pautrizel *et al.* [50] were the first to attempt treatment of pollen and house dust mite allergy by repeatedly applying drops of allergen extracts onto heavily scarified skin. Although he reported the approach to be effective, patients did not tolerate the treatment well. Two years later, Blamoutier *et al.* [51] developed a method for desensitization that would work rapidly

and could therefore be applied immediately before or even during the hay fever season. As Pautrizel *et al.*, before, first performed a scarification of the upper extremity and then applied the pollen extract drop by drop on the scarified skin. After 10–20 min a wheal-like plate formed in the corresponding area. Systemic adverse effects were only rarely observed. In 1962, Eichenberger and Storck [52] also performed this type of co-seasonal desensitization therapy in some of their hay fever and hay asthma patients. Treatment started with the beginning of symptoms and was repeated as soon as the symptoms relapsed. The treatment interval was usually 1 week but ranged between 3 days and 3 weeks. On average, 6–12 treatments were required during every pollen season. A considerable improvement or complete relief was found in 35 of 42 patients that were treated in the first year. In the following 3 years, amelioration of hay fever symptoms was found in 57 out of 72 patients, in 52 out of 60 patients, and in 118 out of 141 patients, respectively. Symptom amelioration lasted for 3 days to 3 weeks. Even patients who responded inadequately to classical or rush desensitization were reported to show a good response [52].

Those clinical studies were performed before the discovery of IgE, when immunology was in its infancy. Although performed with rather large numbers of patients, their studies were neither blinded nor placebo-controlled, and these early examples of epicutaneous allergen-specific immunotherapy (EPIT) with allergens have not been further followed up.

Our clinical experience with epicutaneous allergen-specific immunotherapy

In order to reduce physical damage to the epidermis, we chose to replace scarification with adhesive tape stripping [33,34]. Sticking Scotch (3M Company, St Paul, Minnesota, USA) tape onto the skin and stripping it off repeatedly more gently reduces the cornified layers of the epidermis, thus facilitating the penetration of the allergens into the epidermis and the pulsing of the Langerhans cells, which should then carry the allergen into lymph nodes. We aimed at a treatment duration of 2 or 3 months, as we had previously shown that a 2-month course of allergen-specific immunotherapy which targeted the lymph node was sufficient to induce long-lasting symptom amelioration [53,54].

To assess the safety and efficacy of epicutaneous allergen immunotherapy we conducted a monocentric, placebo-controlled, double-blind trial from March 2006 to December 2007 at the University Hospital Zurich (ZU-SkinSIT-001, NCT00457444). 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. Allergen-treated patients showed significantly decreased scores in nasal provocation tests in the first ($P<0.001$) and second year ($P=0.003$) after treatment. In contrast, placebo-treated patients had decreased scores in the first treatment year ($P=0.03$), but the effect diminished in the second year ($P=0.53$). Although improvement of nasal provocation test scores was not significantly better in the verum group, the overall treatment success was rated significantly higher by the allergen-treated group than by the placebo group. Verum patients reported an approximately 70% improvement of hay fever symptoms, whereas placebo patients reported merely an approximately 20% improvement (2006, $P=0.02$; 2007, $P=0.005$). Therefore, efficacy is at least comparable if not superior to current immunotherapeutic regimens. No severe adverse events were observed. In a few patients we observed the occurrence of eczema under the allergen patch application site, which suggested stimulation of specific T-cell responses. It should be noted that we were actively looking for this eczema and that it was very mild, and it was for no patient a reason to stop treatment. Based on this first clinical experience we conclude that epicutaneous allergen immunotherapy is a promising strategy to treat allergies and merits further investigation [55^{*}], a view shared by Werfel [56].

A phase I/IIa placebo-controlled, double-blind dose-response study to assess the dose, efficacy, and safety of specific allergen immunotherapy in grass pollen allergic subjects by epicutaneous allergen administration was started in 2008 (ZU-SkinSIT-002, NCT00719511) and 2009 (ZU-SkinSIT-003, NCT00777374). A total of 132 and 98 patients in trials 002 and 003, respectively, were enrolled. Study 002 is completing in 2010, and study 003 is expected to complete in 2011. The primary objective is to compare the efficacy of the placebo with that of three different test doses of the epicutaneous pollen allergen administration evaluated by visual analogue scale (VAS) after the treatment (improvement/deterioration of hay fever symptoms). Secondary objectives include comparison of the inhibition of the allergic response assessed by conjunctival provocation test after epicutaneous administration of three different test doses of pollen allergen versus epicutaneous administration of a placebo, comparison of the efficacy of placebo with that of three different test doses of the epicutaneous pollen allergen administration evaluated by weekly VAS during the pollen season 2008 and by VAS before and after the treatment (nasal itching, sneezing, nasal obstruction, rhinorrhea, itchy eyes, lacrimation, itchy ears, itchy palate, lung symptoms), and comparison of the inhibition of the allergic response assessed by skin prick test after epicutaneous administration of three different test doses of pollen allergen and epicutaneous administration of a

placebo. Preliminary results and interim analysis indicate efficacy and safety.

Other research work on epicutaneous/transcutaneous allergen-specific immunotherapy

Using an epicutaneous delivery system (Viaskin, DBV Technologies, Paris, France) that promotes diffusion of allergens through the stratum corneum without prior skin preparation or adjuvant, Mondoulet *et al.* [57*] recently demonstrated in an animal model equivalence of epicutaneous versus subcutaneous immunotherapy. The delivery system consists of an occlusive chamber on the skin which generates moisture and induces the release of the allergens into the skin without systemic diffusion of the intact allergen [58]. This method has been successfully used to treat mice [57*]. Using the same system Dupont *et al.* [59] conducted a bi-center, double-blind, placebo-controlled clinical pilot trial using the Viaskin epicutaneous delivery system to desensitize children suffering from cow's milk allergy. Treatment with verum tended to increase the cumulative tolerated dose of milk, but results failed to reach statistical significance, likely due to low patient numbers. Agostinis *et al.* [60] conducted a prospective, double-blind trial in 30 children with seasonal rhinoconjunctivitis randomized 1:1 to receive treatment with adhesive skin patches containing grass pollen extract (major grass pollen allergens) or placebo mixed 1:1 with vaseline (containing <3% p.v. salicylic acid). The patches were applied for 24h/week for 12 weeks beginning in early February. Rhinoconjunctivitis symptoms were rated and antihistamine use monitored daily. Prick tests were performed before and at the end of the study. Although no statistically significant difference in the prick test reactions was found, the symptom scores (rhinorrhea, nasal obstruction, dyspnea, ocular tearing; $P < 0.044$) and antihistamine consumption were significantly higher in the placebo group.

Conclusion

The problem of allergen immunotherapy is far from resolved, and any improvement of the efficacy or safety of immunotherapy would represent a major breakthrough in treating these highly prevalent diseases. Evidence that epicutaneous allergen immunotherapy is efficacious is accumulating, and it can certainly be further improved by optimizing delivery systems and using appropriate adjuvants. For example, layer-by-layer-assembled multilayer films have recently been shown to successively deliver ovalbumin to Langerhans cells [61], and even more recently, ovalbumin antigen encapsulated in 150 nm polylactate nanoparticles was reported to reach antigen-presenting cells in the epidermis and elicit immune responses in a mouse model [62*]. It is essential that existing data are corroborated with more extensive clinical trial programs.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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- of outstanding interest

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