Review

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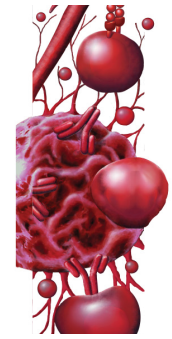
Immunotherapy



Epicutaneous immunotherapy for food allergy as a novel pathway for oral tolerance induction

Epicutaneous immunotherapy is a developing technique, aiming at desensitizing patients with food allergy with less risks that oral ingestion or injection could generate. Several clinical trials have been performed and are currently running, in milk and peanut allergy, assessing the safety of the technique and its efficacy. Preclinical models indicate a major role in the mechanisms of desensitization, for example, Tregs and epigenetic modifications.

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Food allergy is a worldwide issue, with an estimated prevalence of 2–10%, with no treatment currently available. Oral immunotherapy and sublingual immunotherapy have been tested by several authors, in particular for milk, egg and peanut allergy, with significant results in term of desensitization, that is, increase of the dose inducing a reaction during a food challenge, whereas the achievement of sustained unresponsiveness still seems doubtful [1]. The role of skin in allergy has long been considered in terms of sensitization [2–6] and of triggering of acute symptoms, such as urticaria [7] and exacerbation of atopic eczema in IgE-sensitized individuals upon exposure to food antigens [8–12]. Recent data in humans and in animal models that skin may largely be useful to desensitize allergic patients [13] suggesting a major tolerogenic role, for which the cellular or molecular pathways, largely unknown, require in-deep investigation.

The ontogeny of EPIT®

Allergen immunotherapy by subcutaneous administration of an allergen extract in gradual amounts is an effective treatment, with a long-term benefit, for aeroallergen allergy [14], as recognized by WHO [15]. Subcutaneous immunotherapy (SCIT) has other benefits, such as the prevention of new sensitizations in children monosensitized with house dust mite (HDM) [16] and the development of asthma in children with pollen-induced seasonal rhinoconjunctivitis [17].

Skin may be addressed by its outer layer with the allergen deposited onto the skin instead of being injected intradermally. First clinically successful attempts at desensitization date back to the 50s, when Pautrizel *et al.* [18], then Blamoutier *et al.* [19] and Eichenberger and Storck [20] scarified the skin and repeatedly applied drops of extracts of pollen and HDM, until a local reaction appeared. This invasive technique was finally dropped because of it is invasiveness.

More recently, it appeared that an immune reaction may also occur following deposition of an allergen onto intact skin. The local skin immune reaction triggered by diagnostic patch tests was looked for before World War II [21] and further investigated as a model of skin reactivity to HDM in patients with atopic dermatitis (AD) [22]. The so-called atopy patch test [23] proved later on able to show a skin reactivity to food allergens, such as during food allergy associated with AD [24]

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and during digestive manifestations of milk allergy [25]. An important step was taken with the development of a ready-to-use patch test [26], specifically designed to diagnose cow's milk allergy, applied by the parents for their children. The self-application of the patch allowed conceiving a repeated use by patients themselves, thus hopping from a diagnostic device to a therapeutic one.

This observation was the beginning of the so-called epicutaneous immunotherapy, EPIT®, and the development of Viaskin® (DBV Technologies, Paris, France), an epicutaneous delivery system (Figure 1) which allows the allergen to be applied repeatedly onto the intact skin at home, without any subcutaneous injection or instillation through a scarified skin. The technique allows a permanent and long-lasting contact of the allergen with the organism, such as with SCIT or oral immunotherapy (OIT), but without any injection or swallowing of a dangerous allergen, suggesting that a new route for allergen immunotherapy could be considered in the absence of vital risk.

The development of clinical trials

Following those steps, EPIT was first attempted in a proof-of-principle study in patients with food allergy. We conducted a 3-month pilot, double-blind placebo controlled (DBPC) trial in 18 children (age 0.8–7.7 years) with milk allergy [27]. Treatment consisted of three 48-h patch applications on the back (skimmed milk powder as active substance), three-times a week. This first trial showed that EPIT was safe, virtually devoid of serious systemic adverse events (AE), which differs from subcutaneous and oral immunotherapies requiring extensive evaluation and monitoring during therapy and a drop-out rate approaching 20% due to persistent side effects of therapy [28–32]. In SLIT, due to small and heterogenous studies in food allergy [33,34], it was difficult to draw a clear profile of AEs. Recently, the Fleischer *et al.* study exhibit a safer safety profile [35]. Less systemic reactions were notified in SLIT than OIT studies, but SLIT was less effective at least in short-term studies [36–38]. AEs with EPIT were mostly local erythema/eczema occurring at application sites and remaining visible for several days. The estimated risk of local eczema was higher in the active group than in the placebo group (odds ratio: 8.20; 95% CI: 2.72–24.5; $p < 0.001$). The active treatment was thus associated with more frequent complaints for local pruritus and discomfort than placebo, but this did not lead to treatment interruption. Interestingly, local reactions largely varied between patients, even in the active group, but never exceeded local pruritus or local eczema and were easily controlled using local topical medications.

After 3 months, the primary endpoint of the study was the improvement of the cumulative tolerated dose of milk during the food challenges which changed from 1.77 ± 2.98 ml versus 23.61 ± 28.61 ml in the active group versus 4.36 ± 5.87 versus 5.44 ± 5.88 ml with placebo ($p = 0.13$), exhibiting only a trend toward clinical efficacy. Considerable increases were seen in some individuals. This suggested that the treatment duration, 3 months, was too short for most subjects, which is consistent with the kinetics of other immunotherapy techniques [39]. An important point in this pilot trial was that only one child of the active group slightly decreased his cumulative tolerated dose, strongly suggesting that the technique would not aggravate sensitization, such as reported in animal experiments [40]. These results were considered as paving the way to further investigations of EPIT efficacy and suggested that longer treatment periods might be appropriate.

Interestingly, other attempts at desensitization via the epidermis were carried out from a Swiss research group using allergens deposited on a large patch applied on stripped skin: in order to keep epithelial barrier disruption minimal, Senti *et al.* replaced the old skin scarification by a considerably less invasive adhesive tape stripping [41]. The design of the three clinical trials carried out in grass pollen allergy was different from the previous ones [42–45]. The technique comprises a skin preparation with six rounds of skin-stripping with an adhesive tape, and then deposition of the allergen-bearing patch on the stripped skin 12-times for 48 h, in weekly intervals before the pollen season for the first study with a reduction to 8 h of patch application and number of applications for the others. Besides enhancing the penetration of allergens by removing stratum corneum [46], repeated tape stripping is considered by authors as functioning as a 'physical' adjuvant through activation of keratinocytes [47,48]. The first pilot trial revealed that patients treated with pollen extract experienced significant alleviation of hay fever symptoms compared with placebo treated patients [41]. Some systemic side effects, correlated with the degree of stratum corneum disruption, were reported, albeit never severe. The local AEs observed were mild local eczematous reactions under the skin patch [41]. Similar results were observed for the two following clinical trials [45]. The technique is promising but authors insist on the need for further research and development, in order to define an optimal regime, balancing clinical efficacy and safety.

In parallel our group developed EPIT with Viaskin on intact skin in a comprehensive clinical plan for patients with peanut allergy. A Phase 1 safety trial in USA included 80 subjects treated with active treatment (peanut protein) and 20 with placebo (age 6–50 years) [49]. Patients had nonsevere or severe

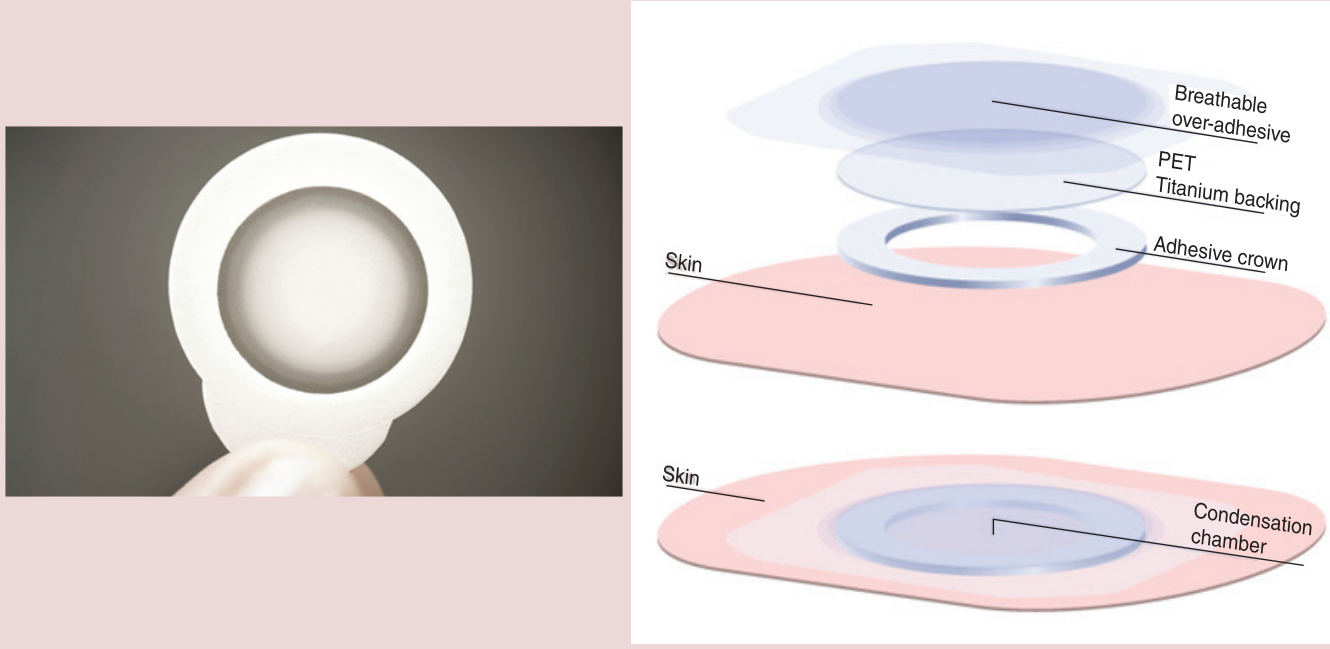

Figure 1 Viaskin, an epicutaneous delivery system on intact skin. (A) Illustrative picture of the device and its dried deposit of antigen on the backing. **(B)** Detailed schematic structure of the device.

peanut allergy based on their history of anaphylactic reactions and tolerated doses of peanut protein per patch were as high as 250 µg in children and 500 µg in adolescents and adults. Overall, 3 of 80 subjects receiving active EPIT and 1 of 20 subjects receiving placebo EPIT dropped out prematurely. In this randomized, DBPC study with 2 weeks of patch application, the peanut patch (Viaskin) proved overall well tolerated and convenient.

The efficacy of peanut EPIT was first investigated in the ARACHILD trial, a DBPC Phase 2A study [50]. Fifty-four children with severe peanut allergy (age 5–17 years) were treated with the peanut patch loaded with 100 µg of peanut protein and DBPC oral food challenges (OFC) were conducted at 6–month intervals over a 18–month period to assess the evolution of the cumulative reactive dose, that is, the cumulated amount of peanut protein triggering a clinical reaction. Enrollment was carried out in highly reactive subjects, that is, those with a baseline cumulative reactive dose <300 mg peanut protein. Success following treatment was defined as ≥ten-fold increase in cumulative reactive dose from baseline and/or a cumulative reactive dose >1 g peanut protein. Children showed consistent and sustained desensitization, with up to 67% responders at 18 months and four subjects reaching 1.1–2.5 g of peanut protein (~4–10 peanuts). Safety data after 18 months were satisfactory and consistent with Phase 1 results. Further to protocol amendment extending the duration of the study, all children were offered 36 months of active treatment (results not yet available).

A large DBPC Phase 2b dose-finding trial [51] involved 22 centers in USA and Europe, enrolled 221 pediatric and adult highly reactive subjects, that is, presenting with a reactive dose ≤300 mg peanut protein during the DBPC OFC. The 12-month treatment allowed comparing the active drug, Viaskin Peanut, loaded with different amounts of peanut protein, to Viaskin placebo. The primary endpoint relied on the comparison of the eliciting dose during the OFC at baseline and after 12 months of treatment: success was defined at 12-month as ≥10-fold increase in eliciting reactive dose from baseline and/or a reactive dose ≥1 g peanut protein. The study was positive: a significant difference was seen at the highest Viaskin dose, 250 µg, with a proportion of responding subjects (50.0%; 28/56 subjects) higher than with placebo (25.0%; 14/56 subjects), p = 0.0108. Viaskin Peanut 250 µg displayed the strongest efficacy in both treatment response rates and evolution of the eliciting doses, compared with Viaskin Peanut 100 µg and 50 µg. Eighteen subjects (32.1%) from the highest dose tolerated equal or more than 1 g peanut protein versus 7 (12.5%) in the placebo group and 23 (41.1%) increased by 10-fold their eliciting dose versus 10 (17.9%). Also, the children subgroup exhibited a higher response rate than the adult and adolescents subgroup, which did not reach statistical significance. Interestingly, a clear dose–response was observed. In good agreement with the clinical observation, a strong

immunomodulation was seen. The active treatment was associated with a robust and sustained increase of median peanut-specific IgG4 titers throughout the study, consistent variations in peanut-specific IgE titers, which increased during the first 3–6 months of treatment then progressively declined toward baseline levels, contrasting with the total absence of variation of the peanut-specific IgG4 and IgE levels in the placebo group. No safety concerns were noted after up to 12 months of peanut EPIT. The most common related reactions were local skin reactions, that is, pruritus, erythema, edema or urticaria at the site of patch application or slightly beyond the patch area. These local skin reactions occurring in 97% of the subjects were mostly mild to moderate in severity. However, these reactions resolved over time and only two subjects (0.9%) dropped out for local AEs (two severe dermatitis). The frequency of occurrence of systemic allergic events, including distal cutaneous reactions, was low (<2%); none of these reactions being severe. Globally, the compliance of the treatment with Viaskin Peanut was high (>95%). Overall, the study showed evidence that EPIT with Viaskin Peanut was both safe and effective (ClinicalTrials.gov identifier: NCT01675882). A follow-up study is currently ongoing with active treatment in all patients, for two additional years, including assessment of clinical tolerance (ClinicalTrials.gov identifier: NCT01955109). The program of Phase 3 studies in children expected to start treatment as of 1 year of age and adolescents/adults should confirm the safety and efficacy profile of Viaskin Peanut across pediatric age range, and in adults it should also provide data on unresponsiveness after a follow-up period without treatment.

Additionally, the Consortium of Food Allergy Research study group has initiated a randomized controlled study of peanut EPIT planned for 30 months of treatment with enrollment of 75 subjects including young children down to 4 years of age (ClinicalTrials.gov identifier: NCT01904604).

At this stage of development, Peanut EPIT is promising. Some caution is needed. When reviewing these results, it is important remembering how they were obtained (doses, population, treatment, duration, definition of primary criteria).

The mechanisms of action

EPIT using the Viaskin device is backed by a deep investigation of its mechanisms of action in animal models.

The proof of concept of EPIT efficacy in animal models

The first experiments in animals showed that EPIT induces a downregulation of Th2-type response to rebalance the Th2/Th1 tones. In a first preclinical proof of concept, mice sensitized to ovalbumin (OVA), peanut or aeroallergens [52] were allocated to 8 weeks of weekly treatment with EPIT and SCIT and compared with untreated and negative control groups. Plethysmography after allergen aerosols showed decreased airway hyperreactivity with EPIT ($p < 0.05$ vs the untreated groups) at levels similar to those seen in the SCIT and negative control groups. Levels of specific IgG2a for all allergens significantly increased with EPIT, similarly to SCIT, whereas the IgE/IgG2a ratio decreased. In a large confirmatory preclinical experiment with peanut-allergic mice, EPIT also reversed airway hyperreactivity measured by the invasive method of resistance/compliance, also similarly to SCIT [53]. In all those experiments, Th2 cytokines, eotaxin and eosinophils counts in bronchoalveolar lavage fluid decreased with EPIT and SCIT ($p < 0.001$ vs sham treatment). In these two studies, no difference was seen between EPIT and SCIT, indicating that EPIT was a good challenger.

In addition to its action on specific IgE, specific IgG2a and organ responses to allergenic stimulation, EPIT appears to act on the allergen-specific cellular response. Tissue eosinophilia is a typical feature of AD, the numbers of eosinophils in the skin usually being modest and correlated to disease severity. Indeed, in the model of OVA-sensitized mice [54], application of allergen onto the skin of sensitized untreated mice induced recruitment of eosinophils. This recruitment significantly decreased after repeated applications of allergen using Viaskin.

Interestingly, the efficacy of EPIT on tissue eosinophilia is not limited to the skin but extends to the digestive organs. A model of eosinophilic gastrointestinal disorder may be obtained with mice sensitized by gavages with whole peanut protein extract and cholera toxin, subsequently exposed to peanuts via a specific regimen [55]. Sustained oral exposure to peanuts in sensitized mice leads to severe esophageal eosinophilia and intestinal villus subatrophia, that is, significantly increased influx of eosinophils into the esophageal mucosa (136 eosinophils/mm^2) and reduced villus/crypt ratios (1.6 ± 0.15). EPIT of sensitized mice significantly reduced Th2 immunological response (IgE response and splenocyte secretion of Th2 cytokines) as well as esophageal eosinophilia (50 eosinophils/mm^2, $p < 0.05$), mRNA expression of Th2 cytokines in tissue – eotaxin ($p < 0.05$), IL-5 ($p < 0.05$) and IL-13 ($p < 0.05$) – and Th2 transcription factor GATA-3 ($p < 0.05$) and intestinal villus subatrophia.

EPIT requires the integrity of the skin

This epicutaneous desensitization process needs the integrity of the skin barrier (Figure 2). A study directly

addressed this issue [56] and it appeared that the immune response generated by Viaskin was strongly influenced by potential alterations of the skin. When Viaskin has been applied on intact skin, the profile of the immune response generated by the treatment was dominantly Th1/Treg whereas, when Viaskin was applied on stripped skin, it was clearly Th2 oriented. This shed a new light on the role of the skin preparation during EPIT and, at least partially, explained why skin has long been considered a route for sensitization rather that for desensitization [3,57–58]. Actually, Strid *et al.* [5,59–60] and Spergel *et al.* [2] demonstrated that the application of antigen on previously stripped skin in naive mice was able to switch antigen-specific T helper cell responses from Th1-type to Th2-type responses. The authors showed that epicutaneous immunization on stripped skin converted an established Th1 response (induced by previous subcutaneous injection with adjuvant) into a Th2 response, as demonstrated by the specific reduction of IFN-γ and IgG2a and the enhancement of IL-4 and IgE. Actually, at least on a mouse model of epicutaneous sensitization, skin seems not to be not inherently sensitizing, and it is likely that factors providing adjuvant activity are required for the development of allergic sensitization to food allergens through the skin [61]. Experimental evidence shows that the hapten picryl chloride applied epicutaneously promotes a Th1 response, whereas following tape stripping of the stratum corneum, skin application stimulates a dominant Th2 response [62].

The immune structures of the skin able to interact with EPIT

The necessary integrity of the upper layers of the skin for EPIT to be efficient underlines the need to take into consideration the skin immune system from its very beginning outer layer.

The immune structures of the skin comprise several strata, which are now the focus of a large investigation [63]. The role of this immune system is to sustain tissue integrity by providing immunoprotection and novel modes of immunoregulation, whereas its dysregulation may promote body surface immunopathologies [64]. EPIT, as opposed other immunotherapy techniques does not need any injection or oral intake of the allergen, which is the basis of its safety. EPIT thus functions according to an original mechanism: the allergen, when deposited onto the skin, without any alteration of it, interacts directly with the immune structure able to detect its presence on the skin. It looks like all the different strata of the skin are involved in this process and thus need to be briefly reviewed [64].

Keratinocytes compose a structural barrier and are strongly involved in the provision and regulation of cutaneous immune responses: keratinocytes are capable of producing and secreting a set of diverse immunological agents and their response may be rapid [64]. The 'inflammasome-type' response to a stimulus increases in the underlying epidermis elevated levels of TNF-alpha, IL-1alpha, IL-1beta and GM-CSF that collectively contribute to the differentiation and activation of Langerhans cells (LC); i.e., intraepidermal dendritic cells (DC)) [64]. Keratinocytes can produce the chemokines, major recruitment signals for systemic immunocytes [65].

Healthy skin and intact epidermal permeability barrier also depends on key a contribution, secretion by keratinocytes of several serine proteases and inhibitors. The serine protease inhibitor, LEKTI, encoded by the SPINK5 gene (5q32), which hereditary mutations cause Netherton syndrome [66] is critically important in barrier function. Filaggrin is a key component of the epidermal differentiation complex of the stratum corneum of human skin at the epidermal layer level. Mutations leading to loss of function of filaggrin have been shown in patients with atopic eczema [67–69] and are associated with an increased risk of allergy [70–73]. Therapies aiming at restoration of barrier function are thus thought to play a role, not only in the effective treatment of atopic eczema, but also in the prevention of further allergic disease development [74].

LCs, compose ~2–4% of epidermal cells [64], in a contiguous network that interdigitates with keratinocytes, largely in the suprabasal layer [75]. LCs may be regarded as epidermal 'trash collectors', clearing the tissue of moieties ranging from toxins through microbes to apoptotic keratinocytes, for which they are equipped with numerous sensors with potent phagocytic and macropinocytotic capacity. In addition, LCs secrete cytokines and other factors, which, in combination with those produced by and/or other myeloid-lineage cells, contribute to the growth and survival of epithelial cells.

As investigated in murine models [76,77], and also in human biopsies [78], dermis contains several identifiable DC subpopulations. They also express microbial sensors and can migrate into the 'stressed' epidermis. They may pick up and processing antigens on route to local LNs [79–81] and also receive antigen by 'hand-off' from LC migrating through the dermis to the LNs. It thus seems that some or all dermal DC may bear some of the functions classically attributed to LC.

LN are secondary lymphoid organs inside which conventional DCs and plasmacytoid DCs reside throughout their life cycle and where they are denoted as lymphoid tissue–resident DCs to distinguish them

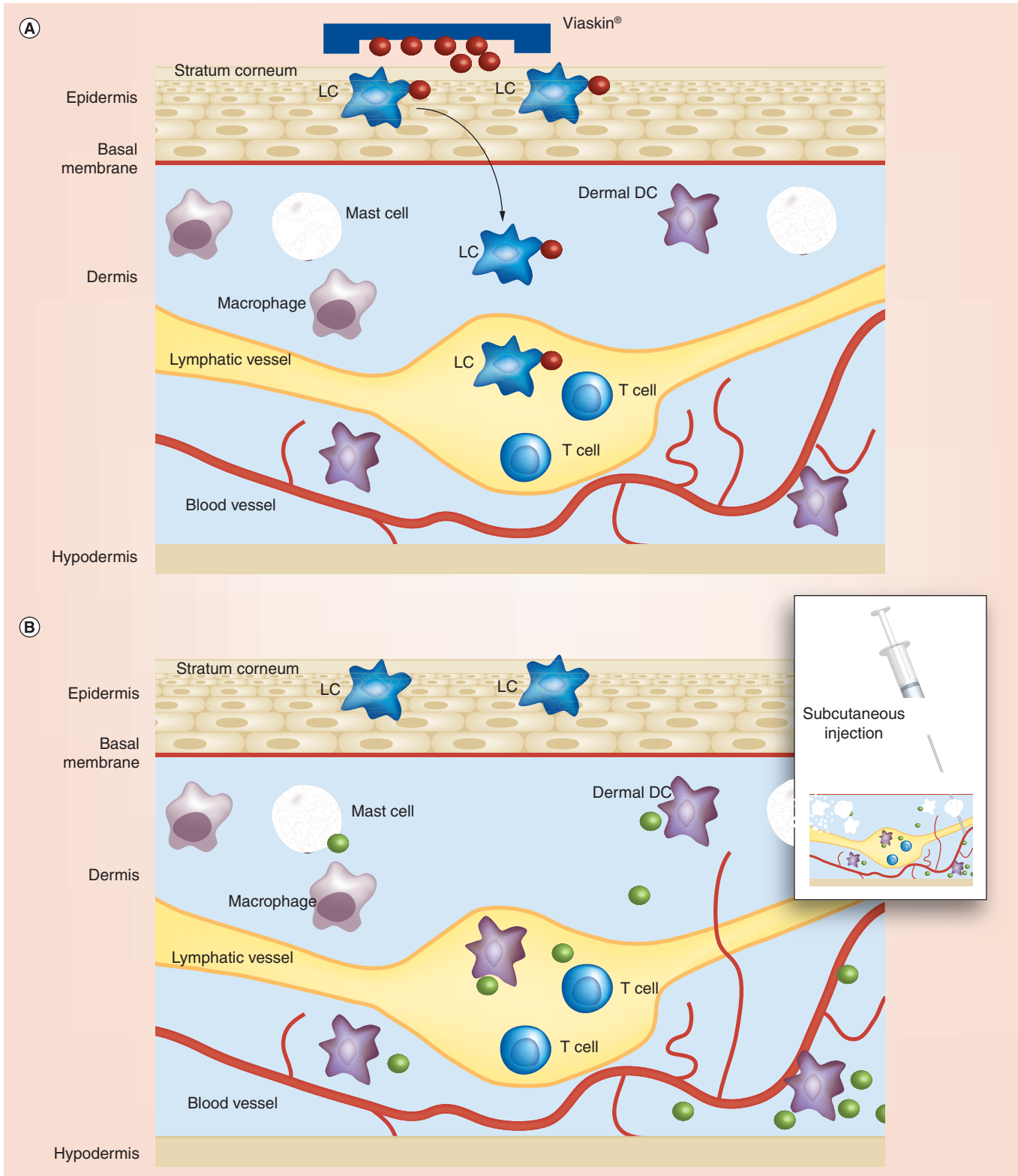

Figure 2. Mechanism of antigen capture by dendritic cells/Langerhans cells. (A) Antigen delivery by Viaskin® from epidermis to draining lymph nodes for epicutaneous immunotherapy. Antigen is delivered with Viaskin into the epidermis. After 6 h, antigen is captured and processed by LC, which migrate to the lymph nodes. **(B)** Antigen delivery by subcutaneous injection. Subcutaneous injections delivered the antigen deeply into the dermis. Antigen is captured by dermal dendritic cells, mast cells and resident B cells. It can passively diffuse into the lymph nodes.
DC: Dendritic cell; LC: Langerhans cell.

from tissue-derived, migratory DCs [82]. CD103(+) DCs have the selective ability to promote *de novo* generation of regulatory T cells via the production of retinoic acid (RA) [83]. RA-producing DCs in the skin LNs seem primarily of the tissue-derived, migratory DC subtype. These RA-producing skin-derived DCs are capable of triggering the generation of regulatory T cells [84].

How EPIT interacts with the skin immune system

The first step in the cascade of events leading to desensitization during EPIT is the capture of the allergen, which is followed by its processing. These different stages of this cascade were studied in animal models, using a Viaskin patch loaded with OVA-labeled with a fluorescent probe. [54]. When thus entering in contact with the intact skin of mice, OVA was neither crossing passively through the skin nor systemically delivered: OVA were locally taken up and internalized by dendritic cells in the superficial layers of the stratum corneum and transported to the draining lymph nodes. This transport was even quicker in sensitized than in naive mice [54].

Immunohistology of the skin after epicutaneous application on intact skin clearly showed that OVA appeared mainly in epidermis and was confined to only few cells in the dermis. The delivery of the native allergen by Viaskin actually allows it to concentrate inside the stratum corneum within the vicinity of immunological cells. There, the allergen is captured by dendritic cells, and detected in draining LNs more than 18 h after the application of the Viaskin patch: this shows the existence of an active immune processing of allergen leading to presentation to the immune system.

The phenotype of cells that capture the allergen in the epidermis and dermis was clearly characterized as DCs expressing langherin receptor (CD207), known as LCs, suggesting that the passage is only mediated by these specialized immune cells. More precisely, these LCs were myeloids DCs and could be divided into at least two subpopulations CD205high, CD86high and CD83high mature DCs and a less mature population of CD205low, CD86low and CD83low. Both express comparably high levels of MHC II, which is crucial for antigen presentation, as well as moderate levels of CD80. CD205 is upregulated during activation of DC, plays a role in antigen uptake, processing and presentation and has been implicated in induction of tolerance [85]. This suggests that both cell subpopulations can present the allergen and modulate immune responses toward a different profile of response, although further studies are required to clarify their precise role.

The role of LCs in the mechanism of the skin-induced tolerance to peanut was investigated in a murine model (Langerin-DTA mice) that constitutively defects in LCs. Skin-induced tolerance after application of peanut proteins to structurally intact skin is abrogated if LCs are absent [86], underlying that LCs play an important role in the induction of tolerance by EPIT.

The role of dermis DCs and events taking place in the LNs may play a role in the induction of tolerance to allergens but is not yet evidenced for epicutaneous immunotherapy.

The generation by EPIT of a specific subset of Tregs

It looks very likely that the core of the desensitization process during EPIT relies on the generation of a specific and probably long-lasting population of Treg cells (Figure 3) [87].

In murine peanut-induced eosinophilic disorders, depleting Treg cells with an anti-CD25 antibody erased the effect of EPIT. Moreover, the protection offered by EPIT-induced Treg cells against peanut oral exposure could be adoptively transferred to sensitized but untreated animals. Tregs are able to migrate to the site of allergen exposure, to induce protection from eosinophil recruitment and Th2-induced inflammation and to induce local Tregs in response to allergen stimulation. EPIT actually proved to be beneficial on the different routes of allergen administration: bronchial hyperresponsiveness [53], eosinophils recruitment in skin [54] and on peanut-induced gut inflammation [55]. Therefore, EPIT induces Tregs, in skin or in draining lymph nodes after LC migration, that are able to recirculate and migrate to different tissues, suggesting the induction of a global desensitization.

This Treg cell population seems long lasting as in the same Treg transfer experiment, the Treg level was not modified 8 weeks after the interruption of EPIT and maintained equivalent suppressive *in vivo* capacity after adoptive transfer into peanut sensitized mice [87]. One clue of the sustainability of the effect was found when the transfer of EPIT-induced Tregs in Foxp3-IRES-mRFP mice induced an increase in mRFP-expressing cells, implying an induction of host Tregs. Activated EPIT-induced Tregs can facilitate Tregs expansion, likely maintaining 'homeostatic' Tregs level.

In this murine model of peanut-induced eosinophilic disorders, it has been evidenced that EPIT increases both spleen and mucosal Foxp3+ Tregs but not IL10+ Tregs (Tr1 population). In contrast with other routes of specific immunotherapy, the suppressive activity of EPIT-induced Tregs does not depend on IL-10

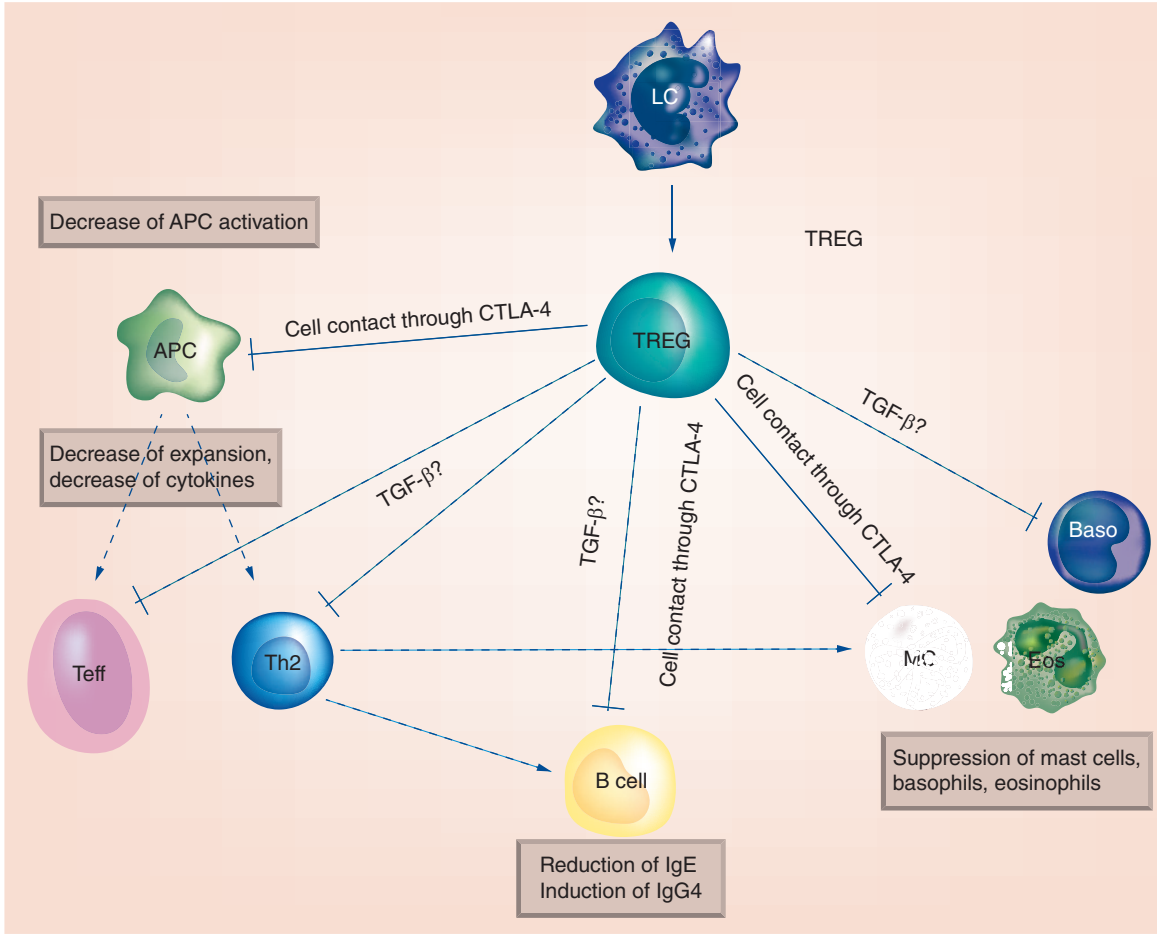

Figure 3. Possible mechanism of action for epicutaneous immunotherapy to treat food allergy.
APC: Antigen presenting cell; Baso: Basophil; DC: Dendritic cell; Eos: Eosinophil; LC: Langerhans cell; MC: Mast cell; Teff: Effector T cell.

but is partly mediated by CTLA-4, probably by cell–cell contact. CTLA-4 has been shown to act in the regulation of hypersensitivity responses to food allergens [88]. Moreover, EPIT may expand Foxp3$^+$ Tregs, of both naive and effector phenotypes. Whereas the naive Tregs subset has been described to preferentially proliferate *in vitro*, effector Tregs display higher suppressive activity *in vivo*, but they are prone to die *in vitro* [89].

Further steps

Many questions may arise concerning this emerging technique and the current research on animal models opens new perspectives.

One question relates to the level of protection offered and more precisely, the protection against the anaphylactic shock, the ultimate risk in food allergy. Investigating this level of protection in a mouse model provides interesting clues. It thus appears that epicutaneous but not oral immunotherapy induces antigen-specific gastrointestinal Tregs and protects against food-induced anaphylaxis [90,91]. This effect might be due to a larger and stronger expression of Tregs gut homing receptors with epicutaneous than with sublingual or oral immunotherapy [92].

The induction of naive Tregs by EPIT could participate in the long-term maintenance as well as in a mechanism of prevention of new sensitizations. Indeed, it was shown that EPIT to a first allergen (milk or HDM) resulted in a protective effect against the second sensitization to irrelevant allergens [90]. These results appeared very consistent and reproducible whatever the role and order attributed to the different allergens according to experiments. This study also suggests a central role for Tregs in the protection against further sensitizations: adoptive transfer of CD4$^+$CD25$^+$ T cells from animal sensitized and treated with EPIT was as effective as EPIT itself in preventing further sensitizations. Tregs act either directly or indirectly at the site of antigen presentation

to create a regulatory milieu that promotes bystander suppression and infectious tolerance [93,94]. The precise role of effector and naive Tregs in this protective process is under investigation. An epigenetic mechanism also seems involved. Indeed, EPIT increased the methylation of the GATA-3 promoter from whole spleen cells and more precisely on $CD4^+$ T cells [95]. This methylation status seems to be long lasting, because it is sustained over at least 2 months [95], probably in relation with the large preventive action of EPIT against further sensitizations. More specific analyses on epigenetic modifications are needed to precisely determine the different steps of immunomodulation. All these mechanisms of action should be confirmed in Clinical Trial to be properly transposed to Human situation. In conclusion, a potential use of EPIT to prevent the development of new sensitizations in allergic children in the appropriate 'window of opportunity' may be considered. Even if AD in children is a risk factor for the development of food allergy and particularly mutations in the FLG gene are the most significant predisposing factor for AD, it was shown in animal models that EPIT keeps efficacy and safety in the presence of FLG mutations.

Conclusion

Following the development of allergen immunotherapy, for which the models remains SCIT, immunotherapy has been investigated using several routes, in the form of OIT and SLIT. SCIT is not applicable in food allergy, OIT seems to alter the quality of life more than the strict elimination diet, with a low level of sustained unresponsiveness at the end of the treatment and SLIT seems to be less effective. The basic novelty of EPIT is its noninvasiveness, avoiding the risk generated by injection or oral ingestion. Interestingly, skin seems to be a powerful organ for desensitization, as proven in animal models and, in humans, at least based on the data available. Further studies are needed to better understand the mechanisms involved, the potency of the technique and its applicability according to ages and the disease to be treated. The development of Viaskin, mostly with an increase dose approach, will be pursue to improve its efficacy in the olders and long-term clinical studies will be required to evaluate the potential role of Viaskin in the atopic march.

Expert commentary

Food allergy has evolved from a period of mere surveillance of patients under elimination diet to a more active handling and the development of immunotherapy. Oral immunotherapy has been attempted by several teams, but was disappointing, despite some efficacy, because of the lack of sustained unresponsiveness in the end and because of an altered quality of life, owing to the risk of an acute reaction to the daily dose, triggered by external factors. EPIT is an alternative route, for which the primary rationale was the total absence of vital risk, owing to the absence of allergen ingestion. Clinical trials are developing and will settle the exact place of the technique in the treatment of food allergy.

Future perspective

EPIT is under heavy investigation, both on the preclinical and on the clinical sides of the technique. Results in animal models are promising and the manoeuvrability of the technique is good, with treatments being feasible at any age, even in young infants, at an age which is the focus of preventive strategies.

Financial & competing interests disclosure

C Dupont declares being the Chairman of the Scientific Advisory Board and a co-founder of DBV Technologies. The other authors work at DBV Technologies. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Epicutaneous immunotherapy (EPIT) is a new technique for immunotherapy, using intact skin as the route for allergen contact with the immune system.
- EPIT avoids any allergen passage into the bloodstream.
- The desensitizing effect of EPIT seems largely born by the generation of allergen specific Tregs.
- EPIT Tregs seem to be long lasting, due to epigenetic changes.
- EPIT might also induce naive Tregs, responsible for the protection against further sensitizations to other allergen.
- Clinical trials are developing and data obtained in peanut allergy indicate a good protection after 12 months of treatment.
- Clinical trials confirm a very good safety profile.
- Based on preclinical results, EPIT might probably be tested in prevention studies.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1 Pratico AD, Leonardi S. Immunotherapy for food allergies: a myth or a reality? *Immunotherapy* 7(2), 147–161 (2015).

2 Spergel JM, Mizoguchi E, Brewer JP*et al.*Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. *J. Clin. Invest.* 101(8), 1614–1622 (1998).

3 Lack G, Fox D, Northstone K, Golding J. Factors associated with the development of peanut allergy in childhood. *N. Engl. J. Med.* 348(11), 977–985 (2003).

4 Strid J, Hourihane J, Kimber I *et al.*Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. *Clin. Exp. Allergy* 35(6), 757–766 (2005).

• **One study that examines the role of epicutaneous exposure to peanut through an impaired skin barrier and the promotion of the risk of peanut sensitization.**

5 Strid J, Callard R, Strobel S. Epicutaneous immunization converts subsequent and established antigen-specific T helper type 1 (Th1) to Th2-type responses. *Immunology* 119(1), 27–35 (2006).

6 Fox AT, Sasieni P, Du TG *et al.* Household peanut consumption as a risk factor for the development of peanut allergy. *J. Allergy Clin. Immunol.* 123(2), 417–423 (2009).

7 Deacock SJ. An approach to the patient with urticaria. *Clin. Exp. Immunol.* 153(2), 151–161 (2008).

8 Werfel T. Skin manifestations in food allergy. *Allergy* 56(Suppl. 67), 98–101 (2001).

9 Charlesworth EN. The allergy and immunology specialist: what is the role in the treatment of skin disease? *Clin. Rev. Allergy Immunol.* 27(2), 123–132 (2004).

10 Wollenberg A, Feichtner K. Atopic dermatitis and skin allergies – update and outlook. *Allergy* 68(12), 1509–1519 (2013).

11 Brough HA, Liu AH, Sicherer S *et al.* Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy. *J. Allergy Clin. Immunol.* 135(1), 164–170 (2015).

12 Hauk PJ. The role of food allergy in atopic dermatitis. *Curr. Allergy Asthma Rep.* 8(3), 188–194 (2008).

13 Jones SM, Burks AW, Dupont C. State of the art on food allergen immunotherapy: oral, sublingual, and epicutaneous. *J. Allergy Clin. Immunol.* 133(2), 318–323 (2014).

• **Overview addressing the need of food immunotherapy and the progress of 3 major routes of treatment: oral, sublingual and epicutaneous.**

14 Durham SR, Walker SM, Varga EM *et al.* Long-term clinical efficacy of grass-pollen immunotherapy. *N. Engl. J. Med.* 341(7), 468–475 (1999).

15 Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J. Allergy Clin. Immunol.* 102(4 Pt 1), 558–562 (1998).

16 Pajno GB, Barberio G, De LF *et al.* Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. *Clin. Exp. Allergy* 31(9), 1392–1397 (2001).

17 Moller C, Dreborg S, Ferdousi HA *et al.* Pollen immunotherapy reduces the development of asthma in children with seasonal rhinoconjunctivitis (the PAT-study). *J. Allergy Clin. Immunol.* 109(2), 251–256 (2002).

18 Pautrizel R, Cabanieu G, Bricaud H, Broustet P. [Allergenic group specificity & therapeutic consequences in asthma; specific desensitization method by epicutaneous route]. *Sem. Hop.* 33(22), 1394–1403 (1957).

19 Blamoutier P, Blamoutier J, Guibert L. [Treatment of pollinosis with pollen extracts by the method of cutaneous quadrille ruling]. *Presse Med.* 67, 2299–2301 (1959).

20 Eichenberger H, Storck H. Co-seasonal desensitization of pollinosis with the scarification-method of Blamoutier. *Acta Allergol.* 21(3), 261–267 (1966).

21 Rostenberg A, Sulzberger MB. Some results of patch tests: a compilation and discussion of cutaneous reactions to about five hundred different substances, as elicited by over ten thousand tests in approximately one thousand patients. *Arch. Dermatol. Syphilol.* 35(3), 433–454 (1937).

22 Mitchell EB, Crow J, Chapman MD*et al.*Basophils in allergen-induced patch test sites in atopic dermatitis. *Lancet* 1(8264), 127–130 (1982).

23 Ring J, Darsow U, Gfesser M, Vieluf D. The 'atopy patch test'in evaluating the role of aeroallergens in atopic eczema. *Int. Arch. Allergy Immunol.* 113(1–3), 379–383 (1997).

24 Isolauri E, Turjanmaa K. Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis. *J. Allergy Clin. Immunol.* 97(1 Pt 1), 9–15 (1996).

25 De Boissieu D, Waguet JC, Dupont C. The atopy patch tests for detection of cow's milk allergy with digestive symptoms. *J. Pediatr.* 142(2), 203–205 (2003).

26 Kalach N, Soulaines P, de BD, 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.* 116(6), 1321–1326 (2005).

27 Dupont C, Kalach N, Soulaines P*et al.*Cow's milk epicutaneous immunotherapy in children: a pilot trial of safety, acceptability, and impact on allergic reactivity. *J. Allergy Clin. Immunol.* 125(5), 1165–1167 (2010).

28 Nelson HS, Lahr J, Rule R *et al.*Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *J. Allergy Clin. Immunol.* 99(6 Pt 1), 744-751 (1997).

•• **Interesting clinical monitoring that evidence the safety concerns of subcutaneous immunotherapy.**

29 Hofmann AM, Scurlock AM, Jones SM *et al.* Safety of a peanut oral immunotherapy protocol in children with peanut allergy. *J. Allergy Clin. Immunol.* 124(2), 286–291 (2009).

•• **Oral immunotherapy creates a lot of debate as severe reactions occurred in the different phases of the study even if mostly in the escalation phase.**

30 Blumchen K, Ulbricht H, Staden U *et al.* Oral peanut immunotherapy in children with peanut anaphylaxis. *J. Allergy Clin. Immunol.* 126(1), 83–91 (2010).

31 Varshney P, Jones SM, Scurlock AM *et al.* A randomized controlled study of peanut oral immunotherapy: clinical desensitization and modulation of the allergic response. *J. Allergy Clin. Immunol.* 127(3), 654–660 (2011).

32 Wasserman RL, Factor JM, Baker JW *et al.* Oral immunotherapy for peanut allergy: multipractice experience with epinephrine-treated reactions. *J. Allergy Clin. Immunol. Pract.* 2(1), 91–96 (2014).

•• **This experience concludes that comparing the rate of epinephrine use in oral immunotherapy treated patients with the rate in untreated patients, the treated patients have approximately a two-fold increased chance of a reaction that requires epinephrine than an untreated patient.**

33 Mempel M, Rakoski J, Ring J, Ollert M. Severe anaphylaxis to kiwi fruit: immunologic changes related to successful sublingual allergen immunotherapy. *J. Allergy Clin. Immunol.* 111(6), 1406–1409 (2003).

34 De Boissieu D, Dupont C. Sublingual immunotherapy for cow's milk protein allergy: a preliminary report. *Allergy* 61(10), 1238–1239 (2006).

35 Fleischer DM, Burks AW, Vickery BP *et al.* Sublingual immunotherapy for peanut allergy: a randomized, double-blind, placebo-controlled multicenter trial. *J. Allergy Clin. Immunol.* 131(1), 119–127 (2013).

36 Chin SJ, Vickery BP, Kulis MD *et al.* Sublingual versus oral immunotherapy for peanut-allergic children: a retrospective comparison. *J. Allergy Clin. Immunol.* 132(2), 476–478 (2013).

37 Keet CA, Frischmeyer-Guerrerio PA, Thyagarajan A *et al.* The safety and efficacy of sublingual and oral immunotherapy for milk allergy. *J. Allergy Clin. Immunol.* 129(2), 448–455, 455 (2012).

38 Burks AW, Wood RA, Jones SM *et al.* Sublingual immunotherapy for peanut allergy: long-term follow-up of a randomized multicenter trial. *J. Allergy Clin. Immunol.* 135(5), 1240–1248 (2015).

39 Marogna M, Spadolini I, Massolo A *et al.* Long-lasting effects of sublingual immunotherapy according to its duration: a 15 year prospective study. *J. Allergy Clin. Immunol.* 126(5), 969–975 (2010).

40 Bauch C, Kolle SN, Fabian E *et al.* Intralaboratory validation of four in vitro assays for the prediction of the skin sensitizing potential of chemicals. *Toxicol. In Vitro* 25(6), 1162–1168 (2011).

41 Senti G, Graf N, Haug S *et al.* Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. *J. Allergy Clin. Immunol.* 124(5), 997–1002 (2009).

42 Senti G, von MS, Kundig TM. Epicutaneous immunotherapy for aeroallergen and food allergy. *Curr. Treat. Options Allergy* 1, 68–78, eCollection 2014 (2013).

43 Senti G, von MS, Kundig TM. Epicutaneous allergen administration: is this the future of allergen-specific immunotherapy? *Allergy* 66(6), 798–809 (2011).

44 Senti G, von MS, Tay F *et al.* Determinants of efficacy and safety in epicutaneous allergen immunotherapy: summary of three clinical trials. *Allergy* 70(6), 707–771 (2015).

45 Senti G, von MS, Tay F *et al.* Epicutaneous allergen-specific immunotherapy ameliorates grass pollen-induced rhinoconjunctivitis: a double-blind, placebo-controlled dose escalation study. *J. Allergy Clin. Immunol.* 129(1), 128–135 (2012).

46 Dickel H, Goulioumis A, Gambichler T *et al.* Standardized tape stripping: a practical and reproducible protocol to uniformly reduce the stratum corneum. *Skin Pharmacol. Physiol.* 23(5), 259–265 (2010).

47 Dickel H, Gambichler T, Kamphowe J *et al.* 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* 63(4), 215–222 (2010).

48 Nickoloff BJ, Naidu Y. Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin. *J. Am. Acad. Dermatol.* 30(4), 535–546 (1994).

49 Agbotounou W, Martin L, Dupont B *et al.* Epicutaneous Immunotherapy (EPIT) is safe for the treatment of peanut allergy in allergic patients. *J. Allergy Clin. Immunol.* 131(2), Abstract 91 (2013).

50 Dupont C, Bourrier T, De Blay F *et al.* Peanut Epicutaneous Immunotherapy (EPIT) in peanut-allergic children: 18 months treatment in The Arachild study. *J. Allergy Clin. Immunol.* 133(2), Abstract 102 (2014).

51 Sampson HA, Agbotounou W, Thébault C *et al.* Epicutaneous Immunotherapy (EPIT) is effective and safe to treat peanut allergy: a multi-national double-blind placebo-controlled randomized Phase IIb trial. *J. Allergy Clin. Immunol.* 135(2), AB390 (2015).

52 Mondoulet L, Dioszeghy V, Ligouis M *et al.*Epicutaneous immunotherapy on intact skin using a new delivery system in a murine model of allergy. *Clin. Exp. Allergy* 40(4), 659–667 (2010).

• **Epidermal delivery system showed an efficacy equivalent to subcutaneous immunotherapy for prevention of allergic airway reactions in a sensitized murine model using inhalative allergen challenge.**

53 Mondoulet L, Dioszeghy V, Vanoirbeek JA *et al.*Epicutaneous immunotherapy using a new epicutaneous delivery system in mice sensitized to peanuts. *Int. Arch. Allergy Immunol.* 154(4), 299–309 (2011).

• **In mice model peanut epicutaneous immunotherapy was promising.**

54 Dioszeghy V, Mondoulet L, Dhelft V *et al.* Epicutaneous immunotherapy results in rapid allergen uptake by dendritic cells through intact skin and downregulates the allergen-specific response in sensitized mice. *J. Immunol.* 186(10), 5629–5637 (2011).

• **With epicutaneous immunotherapy, antigen captured via intact skin by Langerhans cells and brought to regional lymph nodes downregulates the allergen-specific response in previously sensitized mice.**

55 Mondoulet L, Dioszeghy V, Larcher T *et al.* Epicutaneous immunotherapy (EPIT) blocks the allergic esophago-gastro-enteropathy induced by sustained oral exposure to peanuts in sensitized mice. *PLoS ONE* 7(2), e31967 (2012).

56 Mondoulet L, Dioszeghy V, Puteaux E *et al.* Intact skin and not stripped skin is crucial for the safety and efficacy of peanut epicutaneous immunotherapy (EPIT) in mice. *Clin. Transl. Allergy* 2(1), 22 (2012).

- **Decribes the crucial role of intact skin for efficacy and safety of epicutaneous immunotherapy.**

57 Hill DJ, Hosking CS. Food allergy and atopic dermatitis in infancy: an epidemiologic study. *Pediatr. Allergy Immunol.* 15(5), 421–427 (2004).

58 Lack G. Epidemiologic risks for food allergy. *J. Allergy Clin. Immunol.* 121(6), 1331–1336 (2008).

59 Strid J, Thomson M, Hourihane J *et al.* A novel model of sensitization and oral tolerance to peanut protein. *Immunology* 113(3), 293–303 (2004).

60 Strid J, Strobel S. Skin barrier dysfunction and systemic sensitization to allergens through the skin. *Curr. Drug Targets Inflamm. Allergy* 4(5), 531–541 (2005).

61 Dunkin D, Berin MC, Mayer L. Allergic sensitization can be induced via multiple physiologic routes in an adjuvant-dependent manner. *J. Allergy Clin. Immunol.* 128(6), 1251–1258 (2011).

62 Kondo H, Ichikawa Y, Imokawa G. Percutaneous sensitization with allergens through barrier-disrupted skin elicits a Th2-dominant cytokine response. *Eur. J. Immunol.* 28(3), 769–779 (1998).

63 Egbuniwe IU, Karagiannis SN, Nestle FO, Lacy KE. Revisiting the role of B cells in skin immune surveillance. *Trends Immunol.* 36(2), 102–111 (2015).

64 Strid J, Tigelaar RE, Hayday AC. Skin immune surveillance by T cells – a new order? *Semin. Immunol.* 21(3), 110–120 (2009).

- **Skin-expressed antigens for dendritic epidermal T cells in mice contribute to Th2 cell polarization.**

65 Tokura Y, Kobayashi M, Kabashima K. Epidermal chemokines and modulation by antihistamines, antibiotics and antifungals. *Exp. Dermatol.* 17(2), 81–90 (2008).

66 Chavanas S, Bodemer C, Rochat A *et al.* Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat. Genet.* 25(2), 141–142 (2000).

67 Palmer CN, Irvine AD, Terron-Kwiatkowski A *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat. Genet.* 38(4), 441–446 (2006).

68 Thyssen JP, Kezic S. Causes of epidermal filaggrin reduction and their role in the pathogenesis of atopic dermatitis. *J. Allergy Clin. Immunol.* 134(4), 792–799 (2014).

69 Thyssen JP, Tang L, Husemoen LL *et al.* Filaggrin gene mutations are not associated with food and aeroallergen sensitization without concomitant atopic dermatitis in adults. *J. Allergy Clin. Immunol.* 15, 10 (2015).

70 Brown SJ, Asai Y, Cordell HJ *et al.* Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. *J. Allergy Clin. Immunol.* 127(3), 661–667 (2011).

71 Irvine AD, McLean WH, Leung DY. Filaggrin mutations associated with skin and allergic diseases. *N. Engl. J. Med.* 365(14), 1315–1327 (2011).

72 Brough HA, Simpson A, Makinson K *et al.* Peanut allergy: effect of environmental peanut exposure in children with filaggrin loss-of-function mutations. *J. Allergy Clin. Immunol.* 134(4), 867–875 (2014).

73 Venkataraman D, Soto-Ramirez N, Kurukulaaratchy RJ *et al.* Filaggrin loss-of-function mutations are associated with food allergy in childhood and adolescence. *J. Allergy Clin. Immunol.* 134(4), 876–882 (2014).

74 Heimall J, Spergel JM. Filaggrin mutations and atopy: consequences for future therapeutics. *Expert. Rev Clin. Immunol.* 8(2), 189–197 (2012).

75 Maurer D, Stingl G. Langerhans cells. In: *Dentritic cells.* Lotze MT, Thomson AW (Eds). 35–50 (2011). http://store.elsevier.com

76 Henri S, Guilliams M, Poulin LF *et al.* Disentangling the complexity of the skin dendritic cell network. *Immunol. Cell. Biol.* 88(4), 366–375 (2010).

77 Guilliams M, Henri S, Tamoutounour S *et al.* From skin dendritic cells to a simplified classification of human and mouse dendritic cell subsets. *Eur. J. Immunol.* 40(8), 2089–2094 (2010).

78 Ochoa MT, Loncaric A, Krutzik SR *et al.* "Dermal dendritic cells" comprise two distinct populations: $CD1^+$ dendritic cells and $CD209^+$ macrophages. *J. Invest. Dermatol.* 128(9), 2225–2231 (2008).

79 Bursch LS, Wang L, Igyarto B *et al.* Identification of a novel population of $Langerin^+$ dendritic cells. *J. Exp. Med.* 204(13), 3147–3156 (2007).

80 Ginhoux F, Collin MP, Bogunovic M *et al.* Blood-derived dermal langerin+ dendritic cells survey the skin in the steady state. *J. Exp. Med.* 204(13), 3133–3146 (2007).

81 Poulin LF, Henri S, de BB *et al.* The dermis contains langerin+ dendritic cells that develop and function independently of epidermal Langerhans cells. *J. Exp. Med.* 204(13), 3119–3131 (2007).

82 Merad M, Ginhoux F, Collin M. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. *Nat. Rev. Immunol.* 8(12), 935–947 (2008).

83 Guilliams M, Crozat K, Henri S *et al.* Skin-draining lymph nodes contain dermis-derived $CD103(^-)$ dendritic cells that constitutively produce retinoic acid and induce $Foxp3(^+)$ regulatory T cells. *Blood* 115(10), 1958–1968 (2010).

84 Raverdeau M, Mills KH. Modulation of T cell and innate immune responses by retinoic Acid. *J. Immunol.* 192(7), 2953–2958 (2014).

85 Bonifaz L, Bonnyay D, Mahnke K *et al.* Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral $CD8^+$ T cell tolerance. *J. Exp. Med.* 196(12), 1627–1638 (2002).

86 Yu X, Chen T, Feg H *et al.* Oral abstract sessions: langerhans cells are crucial in the skin-induced tolerance to peanut. *Allergy* 69, 1–96 (2014).

87 Dioszeghy V, Mondoulet L, Dhelft V *et al.* The regulatory T cells induction by epicutaneous immunotherapy is sustained and mediates long-term protection from eosinophilic disorders in peanut-sensitized mice. *Clin. Exp. Allergy* 44(6), 867–881 (2014).

•• **By targeting the Langerhans cells at the surface of the skin, epicutaneous immunotherapy generates long lasting immune changes through Tregs induction.**

88 van Wijk WF, Hoeks S, Nierkens S *et al.* CTLA-4 signaling regulates the intensity of hypersensitivity responses to food antigens, but is not decisive in the induction of sensitization. *J. Immunol.* 174(1), 174–179 (2005).

89 Huehn J, Siegmund K, Lehmann JC *et al.* Developmental stage, phenotype, and migration distinguish naive- and effector/memory-like CD4$^+$ regulatory T cells. *J. Exp. Med.* 199(3), 303–313 (2004).

90 Mondoulet L, Dioszeghy V, Puteaux E *et al.* Specific epicutaneous immunotherapy prevents sensitization to new allergens in a murine model. *J. Allergy Clin. Immunol.* 135(6), 1546–1557 (2015).

•• **Epicutaneous immunotherapy to a first allergen, milk or house dust mite, resulted in a protective effect against a further sensitization to an irrelevant allergen.**

91 Tordesillas L, Mondoulet L, Benhamou PH *et al.* Epicutaneous but not oral immunotherapy induces antigen-specific gastrointestinal Tregs and protects against food-induced anaphylaxis. *J. Allergy Clin. Immunol.* 135(2), Abstract 226 (2015).

92 Dioszeghy V, Mondoulet L, Wavrin S *et al.* Larger and stronger expression of Tregs gut homing receptors with epicutaneous than with sublingual or oral immunotherapy. *J. Allergy Clin. Immunol.* 135(2), Abstract 159 (2015).

93 Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. *Nat. Immunol.* 9(3), 239–244 (2008).

94 Lui KO, Waldmann H, Fairchild PJ. Embryonic stem cells: overcoming the immunological barriers to cell replacement therapy. *Curr. Stem Cell Res. Ther.* 4(1), 70–80 (2009).

95 Mondoulet L, Tost J, Ligouis M *et al.* Epigenetic changes following epicutaneous immunotherapy in peanut sensitized mice. *J. Allergy Clin. Immunol.* 135(2), Abstract 143 (2015).