

Novel Administration Routes for Allergen-Specific Immunotherapy: A Review of Intralymphatic and Epicutaneous Allergen-Specific Immunotherapy

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- Intralymphatic allergen-specific immunotherapy
- Epicutaneous allergen-specific immunotherapy
- Vaccination route • Intralymphatic vaccination
- Epicutaneous vaccination

The prevalence of allergic diseases, first described by John Bostock at the beginning of the 19th century as *catarrhus aestivus*,¹ has been continually increasing and has actually reached epidemic dimensions.² Initially conceived as a toxin-induced disease,³ the pathophysiologic understanding of allergy fundamentally changed in 1986 with the discovery of immunoglobulin E (IgE).⁴ However, despite such paradigm change with regard to the causative factor of allergy, treatment options have remained

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unchanged for the past 100 years. Allergen-specific immunotherapy (SIT), introduced in 1911 by Leonard Noon,³ still remains the only disease-modifying treatment option.^{5,6} Strikingly, today's state of the art for SIT has not substantially changed and still consists of numerous subcutaneous injections of increasing allergen doses⁷ aiming to restore normal immunity against allergens.⁸ Several immunologic changes have been observed during successful SIT⁶⁻⁸: redirection of the T helper 2 (Th2)-biased allergic immune response toward a Th1-polarized response associated with reduced production of interleukin (IL)-4 and IL-5, and increased secretion of interferon gamma (IFN- γ); stimulation of allergen-specific IgG and IgA with important blocking activity and direct immune-inhibitory function; and, last but not least, generation of regulatory T cells (Tregs) producing IL-10. Yet, it is still a matter of debate which of these immunologic mechanisms is critical for symptom amelioration. Besides ongoing research on the mechanisms underlying SIT, considerable effort is currently put into the development of highly efficient and patient-convenient administration routes of SIT to facilitate its broad application. Today, only 5% of all allergic patients choose to undergo SIT,⁹ even though conventional subcutaneous allergen-specific immunotherapy (SCIT) has proven its efficacy in several randomized controlled trials.¹⁰ The most important reasons for low treatment acceptance and poor treatment adherence¹¹ are the long treatment duration necessitating 50 to 80 physician-administrated injections associated with the risk of systemic allergic side effects.¹¹ The primary objective for the next century of SIT is therefore to increase its attractiveness by enhancing treatment efficacy, reducing treatment duration, and offering needle-free, self-administrable treatment routes.

Recently, the development of novel "vaccine" administration routes has gained increasing interest both for vaccination against infectious disease¹² and for SIT.¹³⁻¹⁵ The development of sublingual allergen-specific immunotherapy, harnessing the specialties of the mucosal immune system, is discussed in the article by Canonica and Passalacqua elsewhere in this issue. In this article, we discuss the rationale and the unique properties and challenges related to intralymphatic allergen-specific immunotherapy (ILIT) and epicutaneous allergen-specific immunotherapy (EPIT) while outlining the current results of preclinical and clinical trials in these fields.

DETERMINANTS OF THE IMMUNE SYSTEM'S REACTIVITY AND THE CHOICE OF THE "VACCINATION ROUTE"

For a rational choice of the vaccination route, it is essential to understand how an immune response is initiated and governed. Therefore, two basic and not mutually exclusive principles held by the two foremost immunologists of the 20th century need to be considered. First is the "*geographic concept of immunogenicity*," brought forward by Rolf Zinkernagel.^{16,17} It is based on the observation that immune responses can be initiated only in secondary lymphoid organs (SLO), including lymph nodes, the splenic white pulp, and organized lymphoid tissue associated with mucosal organs, such as Peyer patches.^{16,17} The outstanding characteristic of SLOs is their functional microarchitecture offering specialized subdivisions helping immune cells to build an efficient effector response.¹⁸ Findings in mice that carry the alymphoplasia (aly/aly) mutation, and therefore lack functional SLOs, underscore this concept.¹⁹ The afferent lymphatic vessel system carries the antigen from the peripheral tissue entry site to the SLOs, which is a crucial requirement for efficient induction of an immune response, as was elegantly demonstrated by skin flap experiments.²⁰ Lymphatic vessels originate in the peripheral tissue as blind-ended porous structures allowing entrance of cells and macromolecules up to 100 to 200 nm in size. In contrast,

proximal lymphatic vessels are impermeable to molecules larger than 5 to 6 nm.²¹ Depending on the antigen size, transport through the lymphatic vessel system mainly occurs as free drainage or as cell-associated transport.²¹ The transporter cells are essentially tissue resident antigen-presenting cells (APCs), which, on activation by danger signals, emigrate out of the tissue by squeezing through openings between overlapping endothelial cells of lymphatic capillaries.²² These APCs have been called the “ultimate messengers” by Polly Matzinger, who introduced the second key principle regarding initiation and governing of immune responses: “*the power of the tissue in determining the effector class.*”²³ This concept is based on the observation that the first trigger for the initiation of an immune response arises in damaged peripheral tissue, which sends activation signals to educate resident APCs to induce a tissue-tailored response. Supporting such concept of APCs as “mailmen” carrying the tissue address is corroborated by the fact that dendritic cells (DCs) from different sites stimulate different types of immune responses.^{24,25} Further support for the hypothesis regarding the role of tissue in tailoring the effector response arises from the observation that different tissues tend to react in a prototypical manner. The reaction pattern of the skin, or more precisely the dermis, consists of a delayed-type hypersensitivity response based on a Th1-type immune activation with secretion of interferon (IFN)- γ and IL-12, which in turn activate macrophages and induce complement-fixing antibodies (IgG2a type in mice). Conversely, the reaction pattern of mucosal sites, such as the gut, is characterized by a noninflammatory immune response characterized by local production of IgA and systemic accumulation of T cells that do not proliferate profusely or produce IFN- γ , but instead secrete IL-4, transforming growth factor β , and IL-10.^{23,26}

Collectively, this indicates that besides antigen structure, dose, and time, the localization of the antigen plays a crucial role, not only for the decision as to whether to initiate an immune response, but also how to shape the response. In conclusion, direct administration of the antigen into the lymph node is likely to increase immunogenicity and to strengthen the immune response. Because the conventional way of antigen arrival via peripheral APCs, the “ultimate tissue messengers,” is circumvented, presumably the response does not show a tissue-imprinted effector class direction. This implies that the choice of the peripheral antigen administration route (subcutaneously, intradermally, epicutaneously, sublingually) not only determines the magnitude of the immune response through the tissue-inherent number of APCs and the density of lymphatic capillaries, but also influences the effector class of the response through tissue-educated APCs.

INTRALYMPHATIC ALLERGEN-SPECIFIC IMMUNOTHERAPY

The long treatment duration associated with conventional SCIT likely impedes broad acceptance,^{9,11} thus the attractiveness of SIT is likely to be increased by considerable reductions in treatment duration. Yet, to this purpose, treatment efficacy needs to be substantially increased. When considering the fact that only small fractions of subcutaneously injected antigen reach the draining lymph nodes,^{14,27} direct intralymphatic injection of the antigen might be a promising strategy to enhance the efficacy of SIT by increasing antigen availability in the SLOs (see the geographic concept of immunogenicity).^{16,17} In the following sections, we first outline the characteristic microstructure of a lymph node before summarizing results of preclinical and clinical trials testing ILIT for the treatment of IgE-mediated allergies.

Structure Impacts Function

Microarchitecture of the lymph node

The highly organized microarchitecture of SLOs is the prerequisite for the efficient induction of an immune response.¹⁸ The close structure-function relationship has been demonstrated in mice deficient in lymphotoxin α 1 β 2, which is crucial for the generation and maintenance of the SLOs' microarchitecture.²⁸ Generally, lymph nodes are organized into 3 zones: (1) an *antigen-sampling zone* consisting of the subcapsular area and the medulla, which are rich in APCs that deliver the antigen to the T-cell and B-cell zones; (2) a *B-cell activation zone* in the cortex of the lymph node, which contains B-cell follicles; and (3) a *T-cell activation zone* in the paracortex of the lymph node. Such organization in specialized subdivisions facilitates the interaction between immune cells and antigen by juxtaposing APCs and lymphocytes in a small volume, thereby increasing the probability of encountering the cognate T cell.¹⁸ Because an individual antigen specificity is represented by only 1 in 10⁷ T cells or B cells, such concentrations of lymphocytes in the SLOs is key to the efficient induction of an immune response.¹⁴ The end products of such interaction are high-affinity neutralizing antibodies, cytokine-producing Th cells, and cytotoxic T cells.

Challenges to intralymphatic "vaccine" administration

Although present throughout the entire human body, lymph nodes are only superficially accessible in the head-neck region and in the groin. In the groin they can be easily detected by ultrasound as hypoechoic nodules with a diameter of approximately 1.0 to 1.5 cm. Because the sensory innervation of lymph nodes is sparse, direct intralymphatic injection using a 28-gauge needle has been demonstrated to be less painful than a peripheral venous puncture.²⁹

Intralymphatic Allergen-Specific Immunotherapy: A Success Story

Back to the roots of intralymphatic immunization

Intralymphatic immunization was first tested as a method to enhance immunogenicity of cancer vaccines.³⁰ Indeed, antitumor responses were observed to be enhanced after direct intralymphatic injection of tumor cells,^{31,32} DNA plasmids,^{33,34} and naked RNA³⁵ in preclinical as well as clinical settings. Additionally, targeted lymph node immunization has been tested as a method to develop a successful HIV vaccine using various peptides.^{36,37} In all these situations, generation of strong immune responses after administration of minute antigen doses were observed to be the key characteristics of intralymphatic immunization.

Preclinical data of intralymphatic allergen-specific immunotherapy

Animal experiments demonstrated that the same observations hold true when intralymphatic immunization is used in the setting of SIT. For induction of equal humoral immune responses, allergen doses could be reduced 100 times when administered directly into the lymph node as compared with the subcutaneous route. Interestingly, intralymphatic immunization did not polarize the immune response but rather generated overall stronger Th1 and Th2 responses as measured by induction of high IgG1 and IgG2a antibodies associated with an overall increase of IL-4, IFN- γ , and IL-10 secretion. Of note, ILIT protected mice from temperature drop in an anaphylaxis challenge model, whereas SCIT did not prevent temperature drop in sensitized mice on intraperitoneal allergen challenge.²⁷

Significant differences in antigen availability in the SLOs after antigen administration via the subcutaneous or intralymphatic route explain the observed efficacy results. Protein amount in the draining lymph node was demonstrated to be 100-fold higher

after intralymphatic injections as compared with subcutaneous injections.²⁷ Confirmatory experiments in humans revealed efficient “antigen-pulsing” of lymph nodes 20 minutes after intralymphatic injections, whereas only small antigen fractions reached the lymph node 25 hours after subcutaneous injection.¹⁴

Clinical trials with intralymphatic allergen-specific immunotherapy

Several clinical trials testing the efficacy of ILIT for the treatment of grass pollen allergy, hymenoptera venom hypersensitivity, and cat dander allergy have provided highly promising results. For grass pollen allergy, a significant increase in allergen tolerance was observed within 4 months after administration of only 3 low-dose intralymphatic injections, whereas 1 year of continuous subcutaneous injections were necessary to obtain the same result. Overall, the cumulative allergen dose could be reduced 1000-fold. Long-term protection, decrease in allergen-specific IgE levels, and reduction in skin prick test sensitivity were similar after ILIT and conventional SCIT. Of note, ILIT proved to be safe, inducing no grade III or IV systemic allergic side effects in contrast to SCIT ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00470457) Identifier NCT00470457).²⁹ Analogous results were obtained in a multicenter trial including patients with grades III and IV bee venom allergy (Senti and colleagues, manuscript in preparation). This year, a large phase II/III trial was initiated in Denmark to assess efficacy and to optimize treatment dose of ILIT for the treatment of grass pollen allergy. First trial results are expected in 2012 ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01166269) Identifier NCT01166269).

Altogether, ILIT allows high therapeutic efficacy with considerably reduced treatment dose and substantially shortened treatment duration. Combined with its good safety profile, ILIT is therefore likely to increase treatment compliance and reduce socioeconomic costs.

Future directions

Anatomic targeting of antigen delivery at the site of immune initiation (ie, into the lymph node) has been demonstrated to significantly enhance immune responses. Combined with molecular targeting of antigen delivery, ILIT might have the potential to bring the ultimate goal of a single-dose allergy vaccine into reach. Molecular targeting has become possible with the engineering of so-called modular antigen transporter (MAT) allergens that precisely deliver the antigen into the major histocompatibility class II (MHC II) antigen-presentation pathway. MAT molecules consist of a *tat*-derived protein translocation domain (mediating cytoplasmic uptake) that is fused to a truncated invariant chain for targeting of the nascent MHC II molecule.^{38,39} In vitro studies confirmed enhanced immunogenicity of MAT fusion allergens, which were able to induce T-cell proliferation at 100-fold lower doses compared with unmodulated recombinant allergens.³⁸ Additionally, MAT fusion molecules were observed to shift the immune response from a Th2 profile toward a Th1 profile in vitro³⁸ as well as in vivo.⁴⁰ Accordingly, MAT fusion molecules significantly enhanced efficacy of ILIT as measured by protection from anaphylactic temperature drop.⁴⁰ Accordingly, a clinical trial using IVN201 (major cat dander allergen Fel d 1 fused to MAT molecule) has recently proved to be safe and highly efficacious in alleviating clinical symptoms of cat dander allergy (Senti and colleagues, manuscript in preparation, [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00718679) Identifier NCT00718679).

EPICUTANEOUS ALLERGEN-SPECIFIC IMMUNOTHERAPY

The need for 50 to 80 injections over 3 to 5 years is a major drawback of conventional SIT, strongly impeding its broad acceptance.^{9,11} For this reason, much effort has been put into the development of needle-free and potentially self-administrable treatment

routes. In light of the potent immune-surveillance function of the skin,^{41,42} the good accessibility, and its proposed intrinsic Th1-type reactivity pattern^{23,26} (see concept of *the power of the tissue in determining the effector class*), epicutaneous allergen-specific immunotherapy (EPIT) has the potential to emerge as a promising needle-free treatment route for IgE-mediated allergies. We first focus on the structure and immunologic function of the skin before depicting the clinical experience with EPIT.

Structure Impacts Function

The structure of the skin

Human skin is composed of 2 compartments: the epidermis and the underlying dermis.⁴² The epidermis is composed of 4 strata (*stratum basale*, *stratum spinosum*, *stratum granulosum*, *stratum corneum*) forming altogether a protection layer that is 50 μm to 150 μm thick.⁴³ The physical barrier function is mainly exerted by the outermost layer, the stratum corneum, which measures 15 μm to 20 μm . It is built of cornified keratinocytes embedded in a lipid-rich matrix^{42,43} that efficiently excludes molecules larger than 500 Da.⁴⁴ Interdispersed between the imbricated arrangement of keratinocytes, which are the predominant cell type of the epidermis, there are sparse specialized cells such as pigment-producing melanocytes and antigen-presenting Langerhans cells (LCs).⁴² Even though accounting for only 3% to 5% of the epidermal cells, LCs cover up to 20% of the skin surface by forming a continuous network with their dendrites.^{43,45} Holding, therefore, an important function as immune sentinels in first-line defense,⁴² these cells are attractive vaccine targets. The nonvascularized epidermis with its “simple histology” is separated by the basement membrane from the “more complex” dermis, which harbors a great diversity of cell types as well as lymphatic and vascular conduits. Beyond fibroblasts, macrophages, and mast cells, the dermis comprises different subsets of dermal dendritic cells (DCs) as well as T cells.^{42,46}

Immunologic function of the skin

The perception of the skin as a “quasi immunologic organ” dates back to 1983, when Streilein⁴⁷ introduced the concept of the skin-associated lymphoid tissue (SALT), conceived as an immunologic entity based on the interplay between keratinocytes, LCs, lymphocytes, and the skin-draining lymph nodes. Although the understanding of the immunologic functions of the skin is increasing,⁴¹ the relative role of different DC subsets and keratinocyte-derived cytokines/chemokines, released upon infection and/or stress, in shaping adaptive immune response is not yet disentangled: are DCs the ultimate immune controllers⁴⁸ or the ultimate tissue messengers?²³ Supporting the former concept, a functional dichotomy has been observed between different skin DC subsets. Whereas epidermal LCs have been demonstrated to hold a key role in induction of CD8⁺ T-cell responses and in shaping a Th2-type/Treg-type response, activation of dermal DC subsets has been found to be essential for B cell class switching and induction of a Th1-type response.^{49,50} In line with such observation, dermal DC subsets were observed to preferentially localize to the B-cell areas of draining lymph nodes, whereas LCs were observed to migrate to the T-cell areas.⁵¹ Alternatively, increasing evidence supports a pivotal role of keratinocytes in governing the adaptive immune responses.^{23,52} It has been proposed that epithelial cells express different molecular mediators under different conditions, thereby dictating the appropriate type of response. Hence, relatively slight stress to the epithelium, such as abrasion (without penetration), induces a predominance of thymic stromal lymphopoietin, IL-25, and IL-33, which in turn instruct noninflammatory Treg or Th2-type responses. Yet, as epithelial damage increases, expression of additional molecules, such as

IL-1 α , IL-6, and tumor necrosis factor (TNF), skew the immune response toward a Th1-type response.⁵² The degree of epithelial damage as the crucial event in determining the effector class response might as well explain the observed dichotomy of different DC subsets: superficial damage induces a “noninflammatory” response transmitted by LCs, whereas deeper epithelial damage induces a “proinflammatory” response that is carried by dermal DC subsets. Studies that enlighten this area would help to rationally design and target epicutaneously administered vaccines.

Challenges to epicutaneous “vaccine” administration

Even though the skin is easily accessible, simple topical application of a vaccine does not typically induce an adequate immune response because of the low permeability of the stratum corneum. Three thousand years ago, when the skin was first used as a vaccination site against smallpox, this challenge was addressed by disrupting the stratum corneum by scratching: so-called *scarification*.¹² Today, this has been replaced by tape stripping⁵³ and other abrasive methods^{54,55} that gently remove the cornified keratinocytes without disruption of the underlying basement membrane. Recent advances in nanotechnology allow the specific targeting of APCs using micro-needle arrays with defined length.^{43,56} Of note, all of these methods are associated with physical irritation of keratinocytes stimulating the secretion of cytokines, which in turn activate the immune system.^{52,57} Alternatively, penetration can be enhanced by skin hydration over a period of at least 4 to 10 hours⁵⁸ (ie, application of an occlusive patch leading to sweat accumulation^{59,60}).

Epicutaneous Allergen-Specific Immunotherapy: In Small Steps to the New Standard of Care?

Back to the roots of “skin vaccination”

Harnessing the special properties of the skin for administration of vaccines has a long history, dating back more than 3000 years, when *variolation* was performed in China and India in an attempt to immunize against smallpox. The method of variolation was performed by scratching crusts from smallpox lesions of convalescing patients onto the skin of healthy individuals. This historical form of epicutaneous vaccination substantially reduced mortality rates of smallpox infections from 30% during natural outbreaks to less than 1%.⁶¹ Despite such early and successful discovery of epicutaneous vaccination, this route did not attract major attention until the beginning of the 21st century, when interest in needle-free vaccination started to increase.^{12,62} Encouraged by the testing of epicutaneous (transcutaneous) vaccination for various infectious diseases,^{55,63,64} as well as for cancer,^{65,66} epicutaneous allergen-specific immunotherapy was rediscovered.^{13,53}

The role of the skin in the development of allergen-specific immunotherapy

Allergen-specific immunotherapy until recently was predominantly administered “under” the skin as “prophylactic hypodermic inoculations”⁶⁷ as described by Noon³ and Freeman,⁶⁷ although several early attempts were made to deliver the pollen vaccination either “into” the skin or “onto” the skin to overcome the main problems associated with subcutaneous injections.

Intradermal allergen-specific immunotherapy

The first records describing administration of the “allergy vaccine” into the skin (taking advantage of the skin’s specialties) date back to 1929. Soon after the introduction of SCIT by Leonard Noon,³ the risk of suffering a “pollen shock” on subcutaneous allergen administration was recognized as a real problem.⁶⁸ Observations that such patients could be safely and successfully treated by intradermal administrations of

pollen extract encouraged Phillips⁶⁹ to launch a study testing this novel route of SIT. Pollen extract was applied “into the substance of the skin, the same as an intradermal test...Such treatment was not only safe but also highly successful, leading to symptom relief in all the 29 patients after administration of less than three doses.”⁶⁹

The birth of epicutaneous allergen-specific immunotherapy

The first report on successful EPIT dates back to 1921, when an asthmatic patient experienced complete symptom relief after administration of horse hair on scarificated skin, a method called *cutiréactions répétées*.⁷⁰ More than a decade later, M. A. Ramirez readopted this method for the treatment of patients allergic to grass pollen.⁷¹ Encouraged by these early successful reports, French allergologists substantially contributed to the revival of EPIT in the middle of the last century.^{72,73} Whereas Pautrizel and colleagues⁷² administered the allergen extract onto a slightly rubbed epidermis, Blamoutier and colleagues^{73,74} applied the allergen drops onto heavily scarificated skin, called *méthode de quadrillage cutané*. Both observed high treatment success; however, with the method of Pautrizel and colleagues,⁷² a large number of applications was necessary until symptom relief. In contrast, the method of Blamoutier and colleagues^{73,74} could be performed co-seasonally, leading to symptom relief or considerable symptom amelioration after 4 treatments on average. Consistently, both reported fewer and milder allergic side effects upon EPIT than observed with SCIT.^{72,73} Several clinical trials were performed in the subsequent years by Eichenberger and Storck⁷⁵ to test this type of co-seasonal desensitization. Consistently, they observed a treatment success rate of more than 80%. Generally, symptom amelioration was obtained rapidly while lasting between 3 days and 3 weeks. On average, 6 to 12 treatments were therefore required during one pollen season to be practically symptom free. Of note, treatment success rates with EPIT were higher than those observed with classical SCIT, and even patients who responded inadequately to conventional SCIT reported considerable symptom improvement under EPIT. Even though, at that time, this French *méthode de quadrillage cutané*,⁷³ was successfully tested from Portugal⁷⁶ to Switzerland,^{75,77} it was buried in oblivion for the second half of the 20th century before its rediscovery at the beginning of the 21st century.

EPIT with tape stripping: a modification of Blamoutier and colleagues' scarification method

Based on such promising “historical” observations, we performed the first randomized placebo-controlled double-blind pilot trial to test clinical efficacy and safety of EPIT in patients allergic to grass pollen (NCT00457444). In this trial, skin scarification (before antigen administration) was replaced by the adhesive tape-stripping method. As previously demonstrated, tape stripping exerts a dual role: first, it gently removes the stratum corneum,⁷⁸ thereby facilitating penetration of the allergen into the epidermis; second, repeated tape stripping has been demonstrated to activate keratinocytes, which then secrete various proinflammatory cytokines, such as IL-1, IL-6, IL-8, TNF- α , and INF- γ , favoring maturation and emigration of DCs to the draining lymph nodes.^{79,80} Following preseasonal and co-seasonal application of 12 patches in total, patients in the verum group reported a statistically and clinically significant 70% improvement of hay fever symptoms compared with 20% improvement in the placebo group. Of note, no severe systemic allergic reactions were observed, although the occurrence of a local eczematous skin reaction was frequently reported.

Encouraged by these results, a second phase I/IIa trial was initiated to find the optimal treatment dose of EPIT. A total of 132 patients were randomized to either the placebo group or to 1 of 3 verum groups, each receiving different concentration

of allergen extract (Senti and colleagues manuscript in preparation, NCT00719511). To confirm efficacy and to gain insight into immunologic changes induced during EPIT, a third trial, including 98 patients, was initiated (NCT00777374). The first results are expected in 2011. Supporting our results, Agostinis and colleagues⁸¹ recently demonstrated efficacy and safety of EPIT when used as a treatment for grass pollen allergy in children. Hay fever symptoms as well as antihistamine use were significantly reduced in the active treatment group.

EPIT with the Viaskin delivery system: the principle of the occlusive chamber

Based on observations from the atopy patch test, which is able to induce an eczematous skin reaction indicating efficient antigen penetration even on nonabraded, non-pretreated skin,⁸² a French group developed EPIT using an occlusive epidermal delivery system.^{59,60,83} Initially developed for diagnostic purposes (Diallertest), Viaskin technology (DBV Technologies, Paris, France) relies on the ability to deliver whole protein molecules to the skin via perspiration, generated under an occlusive chamber.⁵⁹ Whole protein, delivered via such a system, has been demonstrated to accumulate in the stratum corneum,⁸⁴ where it efficiently targets immune cells of the superficial skin layer that rapidly migrate to the draining lymph nodes.⁵⁹

Murine studies, designed to test therapeutic efficacy of the Viaskin epidermal delivery system (EDS), demonstrated equivalence of EPIT and SCIT in preventing allergic airway reactions on inhalative allergen challenge.⁵⁹ Furthermore, EPIT using the Viaskin EDS proved to be an efficacious treatment for food allergy as measured by prevention of mast cell degranulation on oral allergen challenge in a murine model.⁸³ Based on such successful preclinical testing, a clinical pilot trial was launched to test clinical efficacy and safety of EPIT using the Viaskin EDS in children suffering from cow's milk allergy. After a 3-month treatment, a trend toward clinical efficacy was observed in the verum group, showing increased cumulative tolerance of milk in the provocation test. The treatment was well tolerated with no serious systemic allergic reaction; however, a significant increase of local eczematous reactions was observed.⁶⁰ Recently, phase I (NCT01170286) and phase II trials (NCT01197053) have been initiated to substantiate these findings.

Future directions

EPIT has proved its efficacy in animal and human studies. Yet, there is still potential to enhance its clinical efficacy⁸⁵ and to reduce treatment duration and the number of patch applications. Combination of allergen administration with an adjuvant, precise targeting of APCs by using microneedle arrays or encapsulation of allergen into nanoparticles or liposomes might be useful strategies to achieve such a goal.

Historically, the efficacy of SCIT has been significantly enhanced by mixing the allergen extract with the adjuvant alum.⁸⁶ Alum, however, is not very efficient on epicutaneous administration.⁸⁷ Therefore, novel adjuvants, such as cholera toxin (CT),⁸⁸ heat-labile enterotoxin from *Escherichia coli* (LT),⁵⁵ CpG,⁸⁷ or imidazoquinolines,⁸⁹ are currently tested as adjuvants for transcutaneous vaccination against infectious disease or cancer, respectively. CT and LT, however, which have been tested most extensively and successfully as adjuvants for transcutaneous vaccination against various infectious diseases, do not seem ideal in the setting of SIT because of their Th2 skewing potential.^{43,90} Therefore, we recently tested the immune-enhancing and immune-modulatory potential of diphenylcyclopropanone when used as an adjuvant in EPIT (von Moos and colleagues, manuscript in preparation). Precise antigen delivery to the skin's APCs using microneedle arrays with defined needle length might be a promising technique to further increase treatment efficacy, as demonstrated in

recent studies.^{43,56} Last but not least, incorporation of antigen into elastic vesicles or nonelastic nanoparticles might open new possibilities.⁴³

SUMMARY

In light of the increasing prevalence of allergic diseases,² which strongly contrasts the low percentage of patients choosing to undergo SCIT,^{9,15} research during the next century should aim at optimization of current SIT methods to increase its attractiveness. Considering the “*geographic concept of immunogenicity*”^{16,17} and the “*the power of the tissue in determining the effector class response*,”^{23,26} the rational choice of the vaccination route bears the potential to substantially enhance treatment efficacy, thereby reducing treatment dose while ideally offering a needle-free and self-administrable treatment option.

We believe that allergen-specific immunotherapy, no matter whether mediated by blocking antibodies, Th1 upregulation, Th2 downregulation, or induction of Treg, can theoretically be done with one shot. In childhood diseases, one infection with the wild-type virus is sufficient to induce lifelong immunologic changes. Over the past few years, we were able to demonstrate that the immune system has evolved to optimally respond to the threat posed by live pathogens. Pathogen particles are transported into secondary lymphatic organs and internalized by professional APCs that are activated by pathogen-associated molecular patterns, which enhances innate and adaptive immunity.²¹ When we mimic only one characteristic of pathogens, namely efficient drainage into lymph nodes by direct intralymphatic allergen administration, the number of allergen injections could already be reduced to 3. By combining intralymphatic antigen administration with molecular targeting of the antigen presentation pathway or the addition of pathogen-associated molecules as strong adjuvants, the vision of a single-shot allergy vaccine might become true. Even though the history of ILIT is not older than a decade,^{14,29} its ability to significantly reduce treatment dose and treatment duration, as compared with conventional SCIT, has rapidly channeled the way to the first registration study initiated this year. Epicutaneous allergen-specific immunotherapy on the other hand looks back on a long history.¹³ Although the first reports date back 90 years,⁷⁰ it has attracted attention only in recent years.^{13,53} In contrast to ILIT, it has been developed based on empirical evidence, and the rational understanding of the immunologic processes occurring in the skin is only slowly growing. Nevertheless, there is increasing evidence for an important role of epithelial cytokines in determining the polarization of the immune response.^{23,52} Several clinical trials have indeed demonstrated that EPIT has the potential to ameliorate Th2-derived

Box 1

Pros and cons of intralymphatic allergen-specific immunotherapy

Pros

- Reduced number of treatment applications (efficacy after 3 injections)
- Reduced treatment duration (several weeks)
- Fast symptom alleviation (within 1 month)
- Sustained treatment efficacy
- Good safety profile

Cons

- Ultrasound-guided, physician-administrated, intralymphatic injections

Box 2**Pros and cons of epicutaneous allergen-specific immunotherapy****Pros**

- Needle-free administration
- Potential self-administration
- Reduced treatment duration (1 pollen season)
- Fast symptom alleviation (co-seasonal treatment possible)
- Sustained treatment efficacy
- Good safety profile

Cons

- Local eczematous skin reaction

symptoms of allergic diseases^{53,59,60,83}; yet, the exact immunologic mechanism underlying such immune modulation still needs to be elucidated. However, with the increasing understanding of these mechanisms and the adept use of epicutaneously active adjuvants or microneedle arrays, the efficacy of EPIT is likely to be considerably increased in the future. The outstanding characteristic of EPIT is its good safety profile, potentially based on the fact that the allergen accumulates in the nonvascularized stratum corneum, thereby preventing generalized systemic allergic reactions.

Comparing advantages and limitations of ILIT (**Box 1**) and EPIT (**Box 2**) makes it evident that the choice of either of these methods might not be a question of superiority but a matter of individual preference. Although ILIT might be the method of choice for patients seeking high treatment success within a very short time, EPIT might best be suitable for patients suffering from needle phobia and for those seeking a self-administrable treatment option. Another target population might be children, for whom injections represent a big threat leading to poor acceptance of conventional SCIT. Yet, it is especially children who benefit most from SIT,⁹¹ as administration of SIT early in the course of allergic diseases has the potential to stop disease progression to asthma,⁹² which represents a considerable health burden.⁹³ Such reasoning might highlight the development of a preventive patch-based allergy vaccine, accepted as part of the World Health Organization's recommended "early childhood" vaccination program, to conquer the epidemic of the 21st century.

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