

Novel Allergen Immunotherapy Routes

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Opinion statement

Allergic diseases are among the most common human diseases of humans. Approximately 33 % of the total population in industrialized countries may be affected. The immune response towards allergens is regulated by T lymphocytes and characterized by an interleukin (IL)-4, IL-5 and IL-13 dominated Th2 cytokine profile. Allergen-specific immunotherapy (AIT) is the only causative treatment option and able to change the natural course of disease, e.g., to prevent the development of asthma and new sensitizations. Classically, the allergen extract has been applied subcutaneously to the patient. New application methods for delivering the allergen to the patient have been developed in recent years. The intralymphatic delivery of allergens named intralymphatic immunotherapy (ILIT) has been evaluated in clinical trials and was demonstrated to be a highly potent application route with low effort and side effects while having equal efficacy if compared with current standard AIT forms. However, large studies that verify important questions like optimal dose, injection intervals, new allergen forms, use of adjuvants, etc. are still missing. Moreover, it has to be evaluated, whether different indications like rhinitis, asthma, or atopic dermatitis are suitable for ILIT and whether it is useful in children. Epicutaneous immunotherapy (EPIT) is a possible alternative application form. EPIT is minimally invasive and basically consists of the affixation of allergen containing patches to the epidermis over 6 weeks. From the studies performed so far, the authors concluded, that EPIT is safe and efficacious in a dose-dependent manner after six patches only. EPIT is

increasingly attracting attention because of its capacity to offer a safe, needle-free, and potentially self-administrable treatment option for IgE-mediated allergic diseases. AIT is accepted to be the only causative treatment option for allergies. New application routes in ILIT and EPIT may become more important and allow for different delivery methods in the future, however further clinical studies are required and in preparation.

Introduction

The prevalence of IgE-mediated allergies such as allergic rhinitis (AR) has been increasing worldwide. Up to 35 % of the population of Western countries are already affected by it [1].

Allergic diseases are caused by dysregulation of the immune response against per se innocuous products from the environment. Generally, the immune response to allergens is controlled by T lymphocytes. The faulty regulation causes excessive development of an interleukin (IL)-4, IL-5 and IL-13 dominated T_H2 cytokine pattern. Allergies can be treated specifically by administration of the allergen in question, whereby an allergic immune response is to be changed to a normal

immunity.

Allergen-specific immunotherapy (AIT) is the only causal treatment of IgE-mediated allergic diseases. AIT is able to positively influence the basic mechanisms of type-1 allergies and thus influences the natural course of disease. Hence, it can counteract the development of bronchial asthma and new sensitizations in certain parts of a patient population [2, 3].

The immunological mechanism of AIT is not fully understood until today. The repeated application of high allergen doses induces an immunological response consisting of T_H1 cells, regulatory T cells (T_{reg}), and allergen-specific IgG4.

The healthy immune response differs from the allergic by high numbers of specific T_{reg} cells (T_{reg})

The allergic immune response is created by activation of specific T_H2 lymphocytes due to high amounts of IL-4, IL-5, and IL-13, whereby IgE-producing B lymphocytes and effector cells of allergic inflammatory reaction can be generated and activated.

With increasing chronicity of the allergic disease also T_H1 cells producing $IFN\gamma$ and $TNF-\alpha$ are involved in the disease. The T_H cells are directly involved in tissue destruction of the inflamed organ [4, 5].

Recently, also the importance of IL-17 producing (T_H17) cells has been demonstrated in chronic inflammation [6]. They arise in chronic activation of epithelial cells by $TGF-\beta$ and IL-6 and are capable to activate allergic effector cells. T_H17 cells also induce CXC chemokines (e.g., IL-8), which attract neutrophils and extend their survival.

It has long been known that both during AIT and in normal immune response to allergens, T cells that produce large amounts of IL-10 and/or $TGF-\beta$ are formed [7–9]. Through these two regulatory cytokines, proliferation and cytokine production of effector T cells are suppressed. These T cells develop, as well as the other subpopulations, from naive precursors to an independent regulatory T cell population. Therefore, they are referred to as T_{reg} cells. It is the allergen-specific T cells which are characterized by production of immunoregulatory cytokines and weak proliferation. These T cells need, for their regulatory effect, a permanent activation by dendritic (antigen presenting) cells and

contact to the antigen. Accordingly, they express, to a large extent, various activation markers such as the IL-2 receptor CD25. In early stages of development, they are also typically characterized by the transcription factor FOXP3 (forkhead box protein 3) [9]. T_{reg} and FOXP3 need to their development from naive precursors large amounts of antigen and TGF- α . When determining the number of peripheral T_{reg} subsequent to an antigen stimulation of blood lymphocytes from allergic and healthy subjects, it became apparent that immune response of healthy subjects differs from allergic subjects exclusively in a large amount of allergen-specific T_{reg} , in relation to T_H1 and T_H2 cells, and in formation of a specific tolerance [4]. The generation of a large number of peripheral T_{reg} and induction of immunological tolerance to an allergen is therefore the fundamental target of AIT. However, T_{reg} cells are defined only by their function and therefore do not present a consistent population.

A successful AIT is based on development of regulatory T cells and specific tolerance induction

Activation of naive T cells in connection with dendritic cells (DC) may cause expression of both T_H1 and T_H2 as well as T_H17 or T_{reg} cells. A sufficiently large count of T_{reg} cells in relation to effector T cells is necessary in order to maintain immune response in balance. All functional T cell subpopulations, even T_{reg} , arise from the same naive precursors under the influence of various cytokines, and develop into independent subpopulations [10, 11]. The development of different functional T cell types is regulated by cytokines and transcription factors arising therefrom. IL-12 induces expression of T_H1 cells containing transcription factor T-bet. IL-4 induces T_H2 cells containing transcription factor GATA-3 [6, 12]. The corresponding transcription factors are certain markers of the individual T cell subpopulations.

Using large antigen doses in AIT and SLIT, TGF- β specific T_{reg} are induced, as they are necessary for the development of specific immune tolerance [12, 13, 14••, 15]. For induction of tolerance and specific T_{reg} , principally only the antigen-binding site for MHC-II on the T cell receptor is needed. Therefore, immunological activation and tolerance through AIT can be generated by merely using a peptide mixture of immunogenic T cell epitopes [16]. Identical to healthy immune defense, in a successful AIT the misguided allergic immune response is returned in a normal immune response, balanced by T_{reg} . This leads to an allergen-specific tolerance and a high count of IL-10 secreting T_{reg} cells [13, 17]. Tolerance has to be generated over and over again by activation of T_{reg} from a memory pool. In the course of AIT, IL-10 is also produced by B cells, dendritic cells, and monocytes/ macrophages, whereby these cells are involved in the maintenance of the tolerant state [18].

Amount of allergen and bonding strength determine cytokine pattern of T cells

Soluble antigen/allergen must be incorporated by antigen-presenting cells (APC), processed and then, bound as a peptide to the MHC class II molecules

(HLA-DR, DQ, DP), presented to T cells via the specific T cell receptor. Additional binding molecules and receptors (accessory molecules) help to enhance the effective binding strength between APC and T cell in case of weak or low antigen level. A high count of interactions between antigen specific T cells and APC, via MHC-II molecules linked to antigen peptide and via the T cell receptor, is necessary in order to fully activate a T cell. A suboptimal interaction between APC and T cell caused by too low antigen concentration leads to an only partial T cell activation and development of an IL-4 and IL-5 dominated T_H2 cytokine pattern. Obviously, much higher allergen concentrations are required to develop a T_H1 cytokine pattern and T_{reg} producing IL-10. Thereby for the strength of the individual antigen recognition by MHC-II molecules the T cell receptors plays an important role. Indeed, it appears that binding of allergenic T cell peptides is much lower in allergic than in non-allergic individuals. According to chemical law of mass action, which also applies to biological and biochemical reactions [8], this can be offset by higher allergen concentrations. High amounts of allergen, as they are administered during AIT over time, consequently induce preferably a normal immunity with IL-10/TGF- β and IgG4/IgA antibody formation [8, 19, 20]. Allergic and non-allergic individuals basically recognize the same immunogenic determinants (epitopes) in an allergen [20].

Peripheral tolerance is characterized not only by suppressed cell proliferation and cytokine production, but also by their reversibility. As a matter of fact, in the course of AIT, specifically inactivated T cells may be reactivated through treatment with IL-2 and IL-15 or IL-4. In this case, treatment with IL-2 or IL-15 simultaneously with antigen stimulation leads to reactivation of the immune response with formation of a T_H1/T_H0 cytokine pattern and IgG antibody production [13, 18]. In contrast, IL-4 reactivates a T_H2 cytokine pattern from the immunogenic environment in case of antigen contact. Above all, it was found that formation of FOXP3 and T_{reg} is suppressed directly by GATA-3 transcription factor and T_H2 cell expressed IL-4 [10, 21]. This has practical relevance in so far as AIT may be only partly successful in an active polyallergic or atopic condition. Earliest possible AIT will therefore be more successful than a late onset of immunotherapy treatment [22].

Standard immunotherapy

Subcutaneous and sublingual application methods are considered to be current standard in AIT. In general, treatment is carried out outpatient. Recommended monitoring time after AIT injection amounts to half an hour. AIT is usually initiated by frequently repeated allergen doses and then continued with higher allergen doses up to about 3 years. Several controlled prospective studies demonstrated clinical efficacy of AIT [23, 24]. Clinical improvement, often sustained beyond actual treatment period, and significant reduction in drug use [25•] is frequently accompanied by the above specified immunological mechanisms of change in immune response [2, 26, 27]. In recent decades, the efficacy and safety of AIT has been continuously improved, so that incidence of anaphylactic reactions is located between 0.0005 and 0.01 % only [28].

However, this gold standard is associated with significant discomfort and risks for the patient, and high costs too. Despite the above described advantages of subcutaneous AIT, only a small percentage of allergic individuals prefers AIT

over symptomatic treatment with antihistamines and corticosteroids [29]. Many patients find the subcutaneous AIT as being too laborious and time consuming.

In particular, sublingual application form has been established as a possible alternative. In sublingual immunotherapy (SLIT) allergen preparation is administered daily. Depending on preparation and manufacturer, tablets or drops are being dissolved under the tongue either once a day or several times per week. The treatment period usually lasts 2 to 3 years. For preparations with grass pollen, a promising success rate with low side effects has been observed [30].

Another strategy to design treatment more specific and thus more tolerable, implies use of recombinant allergens. Current allergen extracts contain innumerable components which are not relevant for desensitization. Precisely defined recombinant allergen molecules would allow to administer only those allergen molecules to the patient that cause an allergic reaction. There are promising results of a double-blind placebo-controlled study in 2005, wherein allergic individuals were treated with recombinant timothy grass pollen allergen [31].

Two further alternative strategies that promise treatments to be less elaborate, less time consuming and less risky have been developed in recent years at the University Hospital of Zurich and have been tested in initial clinical trials: the intralymphatic and the epicutaneous immunotherapy.

Intralymphatic immunotherapy

The effectiveness of AIT can possibly be raised most effectively when modulation of the reacting allergic immune system takes place directly in the lymph nodes. Results in murine models and early clinical trials have shown that direct allergen injection in subcutaneous located lymph nodes increased effectiveness of AIT significantly [32]. The required injections for desensitization could thus possibly be reduced to three, and allergen dose might be reduced by a factor of 10 to 100. Injections into lymph nodes can be fast and easily performed by ultrasound guidance, resulting pain exposure is comparable to conventional subcutaneous AIT if inguinal lymph nodes are used [33••].

To achieve a successful immunotherapy, dendritic cells, T_H cells, and B cells have to interact especially within the lymph nodes.

In a randomized trial, it was shown that three low-dose intralymphatic injections increased nasal tolerance to grass pollen extract to the same extent as a conventional subcutaneous immunotherapy continued over 3 years with a total of 56 high-dose allergen injections. Similar results were observed regarding symptoms of allergic rhinitis during a pollen season [32, 33••, 34].

Injections were proved to be simple regarding application and almost painless regarding administration. To check the pain intensity, study participants were asked to compare pain caused by venous blood sampling (i.v.) with the pain caused by simultaneous intralymphatic injection (ILIT) on a visual analog scale (VAS). Nociception under ILIT was equal compared with intravenous injections [34]. An analogous study by Hylander et al. [35••] found similar results.

Witten et al. [36] recently published a clinical study, in which the same allergen extract, same dose and same intralymphatic application method was

used. Although this double-blind placebo-controlled (DBPC) trial with 38 patients showed encouraging immunological changes after three or six intralymphatic injections with grass pollen extract (regulatory T cell response and increased IgG4 levels), no improvements in clinical parameters (symptom or medication scores) were observed. In contrast to our study [37••], injections were administered in just 2-week intervals, which are too short in accordance to general recommendations for immunization to develop successive waves of antigen-specific immune responses without interference [38]. Memory B cell formation and affinity maturation need periods of low antigen presence in lymph follicles, so the competition for the antigen can lead to a positive selection of high affinity B cells. Furthermore, pulsatile antigen kinetics, as achieved by monthly injections, tends to a T_H1 -polarized immune response while continuous antigen exposure through frequent injections, more likely tends to cause T_H2 -polarized immune responses [39]. The higher rate of allergic side reactions observed by Witten et al. can also be explained by shorter injection intervals, which do not allow a decay fast enough of the alum-adjuvant caused intranodular inflammation [40•]. However, these authors believe that Senti et al. and Hylander et al. would have achieved less positive results by choosing a different selection of clinical endpoints as recommended by various institutions [41].

Epicutaneous immunotherapy

The idea for the epicutaneous application method is based on the knowledge that the epidermis has a much higher density than the subcutis concerning specialized antigen-presenting Langerhans cells. Specific loading of these cells with antigen should allow a very effective immune activation. For example, contact allergens are phagocytosed by Langerhans cells which then migrate to regional lymph nodes where an immune response is triggered.

Since the epidermis is not vascularized, also risk of systemic allergen spreading and consequently of systemic allergic side effects should be lower using epicutaneous immunotherapy (EPIT).

Although such epicutaneous or transcutaneous immunization has long been considered as alternative method to conventional vaccination in children's diseases or other infections [42, 43], a 2009 performed, double-blind placebo-controlled (DBPC) study so far was the only clinical trial investigating the potential of patches as application form for allergen-specific immunotherapy [37••].

Placebo-controlled double-blind clinical trials [37••]

Thirty-seven patients with a history of allergic rhinitis to grass pollen, a positive skin test (SPT) and a positive nasal provocation test (NPT) were included in the study and randomized. In cooperation with the Kantonsapotheke Zurich, Switzerland, a patch with a perforated chamber was produced. This chamber served as depot for a mixture of grass allergen/placebo and Vaseline [37••].

Over a period of 3 months at weekly intervals, patches were applicated onto patient's upper arm and removed after 48 h. After 6, 12, and 18 months, tests were repeated and symptoms recorded during first and second season. For

treatment of hay fever symptoms, each patient was provided with identic relief medication.

Nasal provocation testing showed a significant improvement after the first year in the verum group, but also in the placebo group. Titrated nasal application of provocation solutions therefore generated less apparent symptoms in most patients who have performed EPIT. In the second year, however, only in the verum group a sustained statistically significant improvement in NPT was shown. After one season, patients estimated symptoms improvement on a scale compared to baseline showing significant improvements of symptoms only in the active treatment group. Incidence of mild local eczema at application site was significantly increased in the active treatment group compared to the placebo group [37••, 44].

In a second DBPC trial $n = 132$ of $n = 157$ screened individuals with a history of allergic grass pollen rhinoconjunctivitis and positive conjunctival provocation test (CPT) were enclosed. They were randomized into four groups each comprising 33 subjects who received six identical patches each with either placebo or 10, 50, or 100 HEP (histamine equivalent prick) allergen extract of six common grass pollen. Patching started at least 4 weeks before start of grass pollen season. Application site was previously pretreated with adhesive tape six times in succession (tape stripping [45]). Patches were removed after 8 h by patients themselves. Simultaneously with start of the pollen season, application of the remaining five patches was started, which were placed at weekly intervals at different locations on the arm.

Patients were monitored after patch application and interviewed by telephone regarding symptoms and/or investigated during visits. Hay fever symptoms were recorded by visual analog scale (VAS), further symptoms and applied medications were tracked by a patient diary. Moreover, CPTs and SPTs were performed. These efficacy parameters were performed before and 4–5 months after treatment as well as in the subsequent grass pollen season (follow up).

Primary endpoint was the change in subjective hay fever symptoms estimated by VAS. Secondary endpoints included weekly VAS ratings of subjective hay fever symptoms during the grass pollen season, the use of relief medication, changes in CPT and SPT, and safety. The survey was conducted according to the guidelines of the “International Conference on Harmonization on Good Clinical Practice” (ICH-GCP) and the Declaration of Helsinki.

Concerning effectiveness, data from 110 patients were analyzed 4–5 months after treatment and from 93 patients in the course of follow-up. Of these 93 patients, 22 patients each were in the placebo and the 10 HEP group, 25 in the 50 HEP group, and 24 in the 100 HEP group. The group's baseline characteristics, in particular clinical parameters, differed only slightly.

In the first year (2008), in the high-dose group grass pollen associated symptoms of allergic rhinitis (AR) were reduced by more than 30 % versus placebo, in second year (2009) symptoms were reduced by 24 % compared to placebo. Subjective improvement of AR symptoms in the subsequent season versus treatment season was statistically significant and dose-dependent. Higher doses were associated with increased adverse drug reactions (ADR), primarily itching, reddening, wheals, or eczema. Eleven systemic adverse events (AE) were mild (grade 1 or 2) but required treatment and led to the premature exclusion from the trial. Dropout rate due to AEs amounted to 8.3 %. There were no serious adverse events (SAEs) reported.

CPT and SPT showed no statistically significant improvements.

Overall, in both surveys EPIT proved to be safe, but not without systemic side effects. The highest IMP dose demonstrated the best clinical benefit, but also the most—mainly local—side effects.

Conclusion

Immunological mechanisms of healthy and allergic immune response are, as well as AIT, determined by activation of certain subpopulations of specific T cells and related cytokine patterns. Suppression of a T_H2 cytokine pattern by T_{reg} cells via IL-10 and/or TGF- β is the critical factor for success of AIT. Precondition is a sufficiently high quantity of single allergen components in the allergen extract used. Chemically or genetically modified allergens, which are not recognized by existing IgE antibodies, may be used to achieve the required high doses. The effect of AIT at first leads to a specific immune tolerance in peripheral T cells associated with suppression of proliferation and cytokine production. Peripheral tolerance arises both in AIT and SLIT and also in natural hyperimmunization. Success of immunotherapy is determined by IL-4 and GATA-3 transcription factor, by suppression of typical T_{reg} transcription factor FOXP3 and thus by development of specific tolerance. A successful AIT therefore can be expected most likely in a normal developed immunity, whereas in a polyallergic, respectively, atopic environment a lasting success of AIT is less assured [9, 13]. High amounts of allergen contribute to development of T_{reg} and immunological tolerance, whereas small amounts support development of allergic T_H2 response. Therefore, from an immunological point of view, cluster or rush immunotherapy schemes, as successfully used in insect venom allergies for a long time, are clearly preferable to conventional therapy. The fact that only large antigen amounts can generate immunological tolerance applies to all forms of allergen administration. Accordingly, also in oral allergen administration like sublingual immunotherapy (SLIT) only high allergen doses are successful [46]. The optimum allergen amount in AIT is not yet fully established. Too small amounts probably will have no side effects but even possibly no immunoregulatory, tolerizing effect [47, 48]. During AIT, allergen administered at high levels for a long time, induces a normal, by IgG4/IgA antibody dominated spectrum.

In induction of specific tolerance, only MHC-II-linked and presented immunogenic peptides recognized by T cells are actively involved. As T cell peptides or chemically or genetically modified, allergens can be administered in required high doses with lower risk of anaphylactic reactions [16, 49]. Precondition for this is the destruction of IgE interfaces and therefore exclusion of IgE-producing B cells as APC [49, 50, 51•].

Taking into account basic mechanisms of immunological antigen recognition, specific activation of T_{reg} and their cytokine-regulated effect on B cells and effector cells of allergic inflammation, AIT can be adapted to the respective specificities and successfully carried out in early stages of a mono- or oligospecific allergy [22, 52•].

Although ILIT represents a highly potent route for immunization and immunotherapy, more studies are still needed to clarify questions of optimization (dosing, injection intervals, allergen preparations, adjuvants, etc.), questions of

indications (e.g., asthma) or target groups (children, adolescents).

To avoid intralymphatic injections and thus adverse immune responses and side reactions, allergen formulations (recombinant allergens, allergen conjugations, etc.) could be developed suitable to pass to lymph nodes via subcutaneous or transcutaneous application. Apart from that, in total, ILIT promises greater efficiency and less risk at the same efficacy as current AIT treatment methods. But there is still lack of large validating trials.

Based on promising results of previous clinical trials, epicutaneous immunotherapy (EPIT) represents a possible future therapy form. EPIT is barely invasive and basically consists of applying six patches over a period of 6 weeks. EPIT thus appears as a patient-friendly AIT with few side effects, little effort, and good efficiency. But there is still no evidence to recommend general use in practice. Improvement potential lies notably in allergen formulation and administration method in order to minimize side effects. Further clinical trials are needed and being planned.

Compliance with ethical standards

The survey was conducted according to the guidelines of the “International Conference on Harmonization on Good Clinical Practice” (ICH-GCP) and the Declaration of Helsinki.

Conflict of interest

L. Klimek (LK) has received research grants for his institution from ALK Abelló (Germany/Denmark), Allergopharma (Germany), Stallergenes (Germany/ France), HAL Allergy (Germany/the Netherlands), Artu Biologicals (the Netherlands), Allergy Therapeutics/Bencard (UK/Germany), Hartington (Spain), Lofarma (Italy), Novartis/Leti (Germany/Spain), GlaxoSmithKline (UK/Germany), Essex Pharma (Germany), Cytos (Switzerland), Curalogic (Denmark), Roxall (Germany), Biomay (Austria), Thermo Fisher (Germany), Circassia (UK), Biotech Tools s.a. (Belgium), and Meda Pharma GmbH (Germany); and/or he has served as an advisor and on speakers' bureaus for some of the aforementioned companies. LK has received travel grants from HAL Allergy (the Netherlands/Germany), Meda (Germany/Sweden), and Allergopharma (Germany), and he is a consultant for Bencard (Germany), Novartis/Leti (Germany), Meda (Germany), ALK Abelló (Germany/Denmark), Allergopharma (Germany), and Boehringer Ingelheim (Germany).

LK is Board Member of the ENT Section of the European Academy of Allergy and Clinical Immunology (EAACI), Vice-President of the German Academy of Allergology and Clinical Immunology, Vice-President German Union of Allergologists, Member of the Board of Directors of the German Society for Otorhinolaryngology HNS. He is co-editor and author of different chapters of the textbook “Allergien bei Kindern und Jugendlichen” (publisher: Schattauer-Verlag, Germany), author of one chapter in “Allergologie” (publisher: Springer, Germany), and author of different chapters in “Allergologie” (publisher: Schattauer-Verlag).

Annette Sperl declares that she has no conflict of interest.

Thomas Kündig declares that he has no conflict of interest.

Gabriela Senti declares that she has no conflict of interest.

Human and animal rights and informed consent

This article does not contain any original data on studies with human or animal subjects performed by any of the authors. For the cited publications that include original data from previous studies of the authors, human rights declarations have been given in the respective publication.

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- Of major importance

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