REVIEW ARTICLE

100 years of hyposensitization: history of allergen-specific immunotherapy (ASIT)

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Abstract

Hundred years ago, Leonhard Noon and John Freeman published their pioneering works on allergen-specific immunotherapy (ASIT) using grass pollen extracts. To honor their contribution to the development of ASIT as the only causal treatment of IgE-mediated allergies, we review the history of ASIT that started with the anecdotal descriptions of ASIT performed by the ancient king Mithridates (132–63 B.C.) and Jenner's development of a cowpox vaccine. Following Noon's and Freeman's first controlled human trials, ASIT was performed by a large number of modalities and with a myriad of pharmacologic preparations. These developments range from early aqueous pollen extracts and whole bee extracts to chemically modified allergens (allergoids) and various recombinant allergens. In addition to allergen-specific immunotherapy, non-specific immune response modifiers have been used in the past or are in the developmental stage. Also, currently many innovative experimental approaches of ASIT are studied in animal models and human *in vitro* systems and will hopefully further broaden the range of allergies that can be treated by ASIT, with enhanced efficacy and further reduced side-effects.

Definition and terminology

Allergen-specific immunotherapy (also called desensitization, hyposensitization, specific immunotherapy) is the administration of slowly increasing doses of specifically relevant allergen in the treatment of IgE-mediated allergic diseases, until a maintenance dosage is achieved or the patient is free of symptoms (1–3).

In general, 1911 is considered the year of birth of this kind of therapy, although already before that similar activities have been begun (Fig. 1) (4, 5).

In history, the term 'desensitization' was slowly replaced by the more modest approach 'hyposensitization' (6). In the 1980s, the term 'immunotherapy' became popular and with 'specific immunotherapy' (SIT) it is the most commonly used term by now. Unfortunately, this is not really correct, since also the use of monoclonal antibodies, e.g. against Immunoglobulin E, is a specific immunotherapy (7). The correct term would be allergen-specific immunotherapy (ASIT) (see Table 1).

Early precursors of ASIT

The principle of allergen-specific immunotherapy has early precursors in history dating back to the antiquity when King Mithridates from Pontos tried to protect himself against poisoning. Obviously, this was a not unusual risk for political leaders in these days. According to Plinius, King Mithridates VI (132–63 B.C.), also called Eupator, had used increased doses of snake venom to make himself immune against the toxin. It is not clear whether this therapy was successful; however, when the Romans conquered his kingdom, he killed himself with the sword (8).

Another and possibly more important route of allergen-specific immunotherapy is the history and development of

bleeding, and then administer salines. In the other 14 cases so treated the condition of the patients permitted of the delay necessary for preparatory treatment and for the removal of all blood clots from the abdomen.

The crisis having passed, and when the case is first seen subsequent to the formation of a distinct and encapsulated hæmatocele, more conservative treatment is warranted. With rest in bed the majority of such cases undergo complete absorption, the only indication for operative interference being the possibility of secondary rupture of the hæmatocele demanding cœliotomy, or infection of the sac, which is best treated by vaginal incision and drainage. Against an entirely expectant line of treatment the element of time has to be considered, especially with hospital patients. Large hæmatoceles may take weeks to undergo complete absorption, which loss of time may be prevented by the safe proceeding of vaginal incision and drainage. Of my six cases so treated five were typical cases of retro-uterine hæmatoceles, and the patients left the hospital within three weeks from date of admission. In the remaining case of hæmatoma, abdominal section having shown that the blood was encapsulated in the broad ligament, the abdomen was closed and the case further treated by vaginal incision and drainage.

Dundee.

PROPHYLACTIC INOCULATION AGAINST HAY FEVER.

BY L. NOON, B.C. CANTAB., F.R.C.S. ENG.

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HAY fever is a form of recurrent catarrh affecting certain individuals during the months of May, June, and July. It is caused by a soluble toxin found in the pollen of grasses. The patients present the idiosyncrasy of being sensitive to this toxin, which is innocuous to normal individuals. The idiosyncrasy may be detected during any season of the year by dropping a little of an extract of grass pollen into the eye of the suspected individual; a reaction, described more fully below, will be obtained in the case of a hay fever patient, but a normal man will show no effect.

Bostock (1819)[1] recognised the seasonal recurrence of hay fever as separating it from other forms of catarrh. Blackley (1873)[2] advanced much evidence in favour of the pollen theory of its causation, but we owe chiefly to Dunbar (1903)[3] the exhaustive scientific proof of this theory. Dunbar showed that not only all the mucous membranes but even the skin of hay fever patients is sensitive to pollen toxin in a way not shown by normal individuals. He also proved that the injection of the pollen toxin gives rise in animals to the production of an antitoxin having a specific power of neutralising this toxin. Further, in hay fever patients, he showed the occurrence of some of the reactions associated with the production of immunity:—namely, a specific precipitation of pollen extracts by the patient's serum, and the phenomenon of complement deviation, during the hay fever season, and persisting for a short time after this. Pollen toxin is, therefore, a body capable of giving rise to the production of antibodies in animals and even in hay fever patients, subjected to its action. It is also undoubted that hay fever patients sometimes become cured of their idiosyncrasy. The most reasonable explanation of this phenomenon would seem to be, that the cured patients have had the good fortune to develop an active immunity against the toxin, to the action of which they have been liable for so long.

The repeated absorption of toxin at short intervals is, however, more likely to induce a condition of hypersensibility, and this is the more usual fate of the patient, who becomes only more sensitive during each succeeding season. The local application of a specific serum, such as pollantin, offers a reasonable method of treatment, but one which is difficult and laborious, and which is not calculated to bring about a permanent cure. Cures are, indeed, ascribed to the use of this remedy, but admittedly in exceptional cases; and where the conditions are not understood and the experience is not constantly repeated, one must hesitate to attribute the result to the cause cited. On general grounds a much more satisfactory result would be expected from the induction of an active immunity, and it seemed worth while to put this expectation to the test of experiment. The questions to be answered are as to what degree of immunity can be induced in hay fever patients by inoculations of pollen toxin, how these inoculations may best be regulated, and whether the affection can by this means be permanently cured.

With this end in view the experiments here described were undertaken in the past autumn, winter, and spring to study the reaction of hay fever patients towards inoculations of pollen toxin. The off season of the year, when the patients were not exposed to spontaneous inoculations, was favourable to this investigation, as the scheme of dosage was then not liable to be upset by spontaneous absorption of toxin from the air, laden with actively poisonous pollen grains. The plan of experiment was to obtain a numerical measure of the sensitiveness of the patients to the pollen toxin and to observe whether this was increased or decreased by subcutaneous inoculations of various quantities of pollen toxin. These observations can be conveniently carried out by the method described below, and it was found that, with well-regulated dosage, it was possible in every case to raise the patient's resistance, to a marked degree, within the lapse of a few months, while, on the other hand, ill-regulated dosage was at once made evident by a decrease in the resisting power.

The pollen extract used was prepared by Dunbar's method of extraction with distilled water, aided by freezing and thawing several times. The extracts were boiled for ten minutes after having been sealed in glass tubes; this treatment was not found to decrease their activity at all. The pollens tested were grass pollens of different species—*Phleum pratense*, *Poa trivialis*, *Holcus lanatus*, and *Agropyrum caninum*. These pollens were all found capable of exciting an energetic reaction when instilled into the conjunctival sac of hay fever patients. Timothy grass (*Phleum pratense*) was found to yield the most active extract, and this extract was consequently used throughout the rest of the experiments. One gramme of pollen was extracted with 50 c.c. of water. The activity of this extract may be judged from the fact that one drop of a five thousand-fold dilution is sufficient to excite a distinct reaction in the conjunctiva of the more sensitive patients.

In order to express the strengths of pollen extracts used in testing patients and the doses of pollen toxin given subcutaneously, a unit of pollen toxin has been arbitrarily chosen. This unit is the quantity of pollen toxin which can be extracted from the thousandth part of a milligramme of Phleum pollen, and it has the advantage that all the quantities used can be expressed in whole numbers. The strength of a pollen extract is given below in terms of the number of such units contained in a cubic centimetre of the extract. Extracts of other pollens have been standardised against the Phleum extract by comparative tests on the eyes of hay fever patients.

A measure of the patient's resistance during the experiments is obtained by observing the strength of pollen extract necessary to excite a conjunctival reaction. One drop of the diluted extract is instilled into the eye. The reaction obtained consists in a reddening of the caruncula and, to a lesser degree, of the palpebral conjunctiva, together with a slight injection of the vessels of the ocular conjunctiva and some lacrymation. The patient experiences a feeling of burning and itching. These signs reach a maximum in about five minutes, and a little later there may be a slight attack of sneezing. The reaction lasts as a rule about half an hour. The strength of the extract, which is just sufficient to give this reaction, is used to describe the resistance of the patient. The most sensitive patients examined gave before treatment a distinct reaction with a dilution containing only 4 units per c.c., their resistance is described as 4; the least sensitive reacted to a strength of 70 units per c.c., or, in other words, had a resistance of 70. Normal individuals fail to react with the strongest extract (strength 20,000 units) and even resist the application of fresh pollen dust to the conjunctiva. Their resistance is therefore, by our scale, more than 20,000, but it is not infinite as a cubic centimetre of this extract injected beneath the skin of a normal man has been

[1] John Bostock: Medical and Chirurgical Transactions, vol. x., 1819, p. 161.
[2] C. H. Blackley: Experimental Researches on the Causes and Nature of Catarrhus Aestivus, London, 1873.
[3] W. P. Dunbar: Zur Ursache und specifischen Heilung des Heufiebers, München, 1903.

Figure 1 Title page of Leonhard Noons original publication, Lancet 1911.

Table 1 Various names for allergen-specific immunotherapy (ASIT)

Desensitization
Hyposensitization
Prophylactic inoculation
Anti-anaphylaxis
Active immunization
Allergy vaccination
Immunotherapy
Specific immunotherapy (SIT)
Allergen-specific immunotherapy (ASIT)

vaccination with the strategy to protect humans against smallpox infection by bringing them in contact with cowpox as it was observed by the British Lady Montague in Turkey and taken up by Edward Jenner who developed the true cowpox vaccine as the first really effective medical prevention program (9). By the way, the first country in the world where smallpox vaccine was introduced obligatory by law was Bavaria in 1807 (10).

Around the turn of the 18th century, Samuel Hahnemann developed the concept of homeopathy, based on the observation that similar substances may do harm, but also may be helpful in the cure (11). So he started to work with a series of dilutions to treat diseases caused by the respective substances. While Samuel Hahnemann might have been at the scientific level of his time, his successors did not continue in the same way, but rather stuck to a kind of 'ideology', exaggerating the dilution calling it 'potency'. Yet the specificity of the idea that one and the same agent induces the disease and is used for cure is similar to the approach of immunotherapy; the clear-cut difference is that ASIT follows a dose–response curve, and its efficacy is proven by many placebo-controlled trials (12–15).

Early attempts at the beginning of the 20th century

In 1903, Dunbar in Hamburg tried to adapt the concept of protective immunity also to the treatment of hay fever (16). First, he showed that a local reaction induced by a pollen grain was totally imitated by the injection of an extract of pollen in alcohol or brine. So Dunbar developed a hyperimmune serum in animals vaccinated with pollen extract to cure hay fever (16, 17). The substance was called 'Pollantin,' and we do not know about larger studies; presumably, there were all kinds of anaphylactic episodes, when patients underwent this treatment.

In Munich and Berlin, A. Wolff-Eisner was engaged in the treatment of hay fever; he realized that proteins in pollen are the elicitors of hay fever. He wrote an exceptional book, but he was without students and his methods were not followed up (18).

In Paris, Alexandre Besredka, in the attempt to neutralize the hypersensitivity induced in animals injected a diluted solution of the allergen and then successively increased the dose (19, 20). With this procedure, he was able to actually induce 'tolerance', which means that the animal was tolerating a dose that was earlier eliciting an anaphylactic episode. He called this procedure 'anti-anaphylaxis' and was probably the first to use allergen-specific immunotherapy in an experimental animal model. A. Besredka furthermore was a very inventive thinker; he believed that anaphylaxis actually was occurring in the brain, an idea that had been forgotten for almost 90 years, when people like John Bienenstock in Hamilton started to bring the concept of psycho-neuro-immunology into allergy, showing that anaphylaxis can be conditioned in a Pawlowian mode (21). Besredka called his treatment 'vaccination anti-anaphylactique' to render the organism tolerant against the effects of the postulated toxin in the pollen (19, 20).

Prior to Dunbar, the US researcher H. Holbrock Curtis had tried to induce immunity by subcutaneous injections of several pollen from flowers and ragweed (22). However, due to significant side-effects, this was not continued.

In the United States, Rosenau and John F. Anderson found that successive doses of foreign serum (horse serum) can produce immunity after repeated injections while small quantities had elicited anaphylaxis (23).

Interestingly, Clemens von Pirquet obviously observed a similar phenomenon that he described in his book 'Serum sickness' when he reported of a self-experiment on Dr. v. P. who received diphtheria and scarlet fever serum in intervals of 6–10 days in the skin to test local skin reactivity and found a slow decrease in the local skin reaction in the sense of Besredka's anti-anaphylaxis (24).

At the same time, the first fatal accident during desensitization in a patient with horse allergy was reported in 1910 who died after injection of small amounts of horse serum with the symptoms of anaphylactic shock (8). In 1909, Scheppegrell administered powder of dried pollen into the nose of patients suffering from hay fever (25).

Noon and Freeman's pioneering work 1911 (Fig. 2A,B)

In 1911, Leonard Noon – probably having read the work and experiments of A. Besredka – had the courage to try this type of treatment in humans. First, he had to prepare extracts of various pollen and find the right dilution in skin tests, before he started to inject patients suffering from hay fever with grass pollen-derived allergen extracts in increasing doses (Fig. 3). To measure the effect, he used conjunctival provocation tests administering droplets of different extract dilutions into the patient's eye and assessing the redness and inflammatory reaction. He did this provocation procedure prior to therapy and after treatment (which he called 'prophylactic inoculation'). His paper published in the Lancet reads like an excellent piece of clinical research work (4).

Noon unfortunately died soon at a very young age from tuberculosis (8). His work was continued by his colleague John Freeman who in the same year published larger observations also in the Lancet 1911 under the title 'Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine' (26). Noon's and Freeman's method was taken up rapidly all over the world by physicians trying to treat allergic diseases.

Robert Cooke, one of the creators of the term 'atopy,' introduced this therapy in the United States calling it 'active

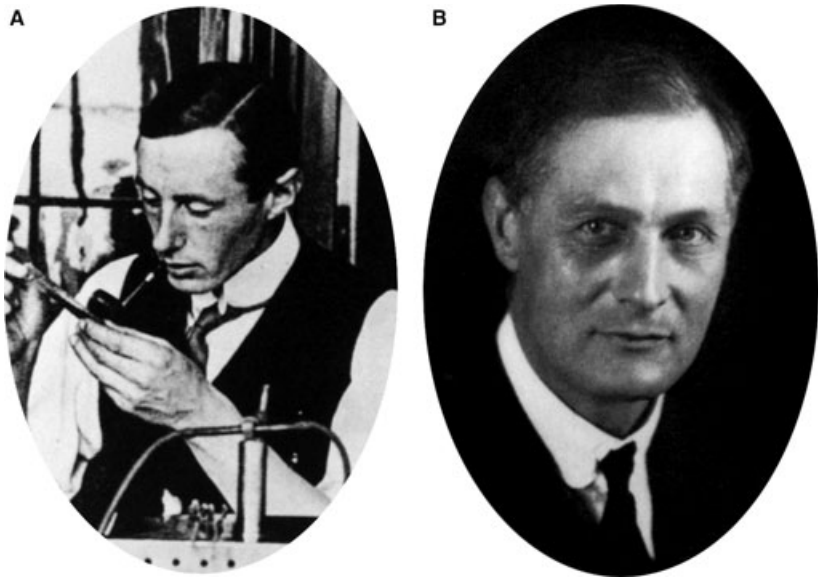

Figure 2 (A) Leonhardt Noon (1878–1911) and (B) John Freeman (1877–1962), the pioneers of allergen-specific immunotherapy.

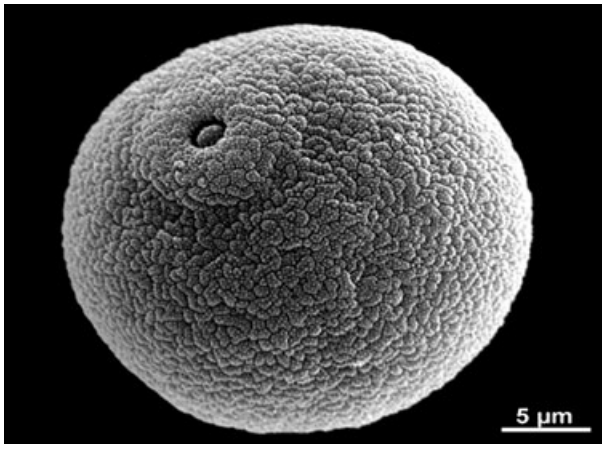

Figure 3 Scanning electron microscopy of a grass pollen grain – Noons therapeutic agent (courtesy from Heidrun Behrendt, Munich).

immunization' in 1914 (27). Later, in 1922, he proposed the term 'hyposensitization' as a better name for this type of treatment (6).

Early days of routine allergen-specific immunotherapy ('hyposensitization')

The pioneers using hyposensitization in the first half of the 20th century knew that the secret of success with this kind of treatment was in the selection and preparation of the right allergen – mostly pollen – extract. Several attempts have been made to produce extracts either by alcoholic (ethanol) or etheric solutions. A. Coca found the recipe, which was taken up by most allergists as Coca's solution for preparing allergen extracts (Table 2) (1).

Table 2 Composition of Coca's solution

NaCl	5.0 g
$NaHCO_3$	2.75 g
Phenol	4.0
Aqua dest. ad	1000.0

The method was used, taken up, and recommended in early allergy textbooks all over the world, without real good clinical studies. This was not the custom in those days.

Treatment of allergy in the first half of the 20th century

It is worthwhile to look into the old textbooks of allergy from the first half of the 20th century (Table 3), where one can find all kinds of treatment modalities for the various allergic diseases (Table 4). Most of these textbooks contain detailed schedules for specific or non-specific desensitization. Table 5 shows the units used for standardization of allergen extracts.

Parallel to the early attempts with allergen extracts, British researchers propagated in 1917 the use of peptone [Witte-peptone (antigens digested with hypochloric acid, pepsin, or trypsin)], which was used in bronchial asthma, migraine, and hay fever. This therapy was also used with epi- or dermal application by the French researchers Pasteur Vallery-Radot and Blamoutier (28).

In 1911, Lambert, Ancel, and Bouin used the term 'Skeptophylaxie' for a phenomenon of transient unresponsiveness after oral application of antigen (29).

'Unspecific' desensitization

After Dale and Laidlaw had shown that histamine was the principal mediator of anaphylaxis, early attempts were

Table 3 Textbooks on allergy from the first half of the 20th century

Author	Year	Title	City
Von Pirquet, C.	1919	Allergie	Berlin
Wolff-Eisner, A.	1910	Die Überempfindlichkeit	Munich
Richet, C.	1911	L'anaphylaxie	Paris
Coca, A. F.	1920	Hypersensitiveness	New York
Kämmerer, H.	1928	Allergische Diathese und allergische Erkrankungen	Munich
Hansen, K.	1928	Allergie	Berlin-Vienna
Doerr R	1929	Allergische Phänomene	Berlin
Wells, H. G.	1929	The chemical aspects of immunity	New York
Urbach, E.	1935	Klinik und Therapie der allergischen Krankheiten	Vienna
Kallos, P., Kallos, L.	1937	Die experimentellen Grundlagen der Erkennung und Behandlung der allergischen Krankheiten	Berlin
Cooke, R.	1947	Allergy in theory and practice	Philadelphia
Vaughan, W.T., Black J. H.	1948	Practice of allergy	St Louis

Table 4 Allergy treatment (before 1950)

Abstinence (avoidance)
Peptone
Vaccines (bacterial, tuberculin, etc.)
Desensitization
Non-specific desensitization (histamine, histaminase, etc.)
Sympathomimetics (ephedrin, amphetamine, adrenaline, etc.)
Parasympathicolytics (Belladonna, atropine, hyoscyamus, stramonium powders, etc.)
Opiates (opium, codeine, cocaine, etc.)
Antihistamines
Expectorants (iodide, etc.)
Hormones (insulin, pituitary and adrenal extracts)
Vitamines and calcium
Hypnotics (urethane, ether, tribromethanol)
X-ray (thorax, spleen)
Surgery (cervical sympathectomy, ganglion, etc.)
Miscellaneous (aspirin, whiskey, etc.)

performed to achieve unspecific desensitization with the use of especially coupled histamine-azoproteins, a trend that was later continued (30, 31) leading to mixtures of low-dose immunoglobulin G and histamine complexes (brand name 'Histaglobin').

In consequence of the so-called procedures in 'non-specific hyposensitization,' the use of microbial or bacterial lysates has to be mentioned which as a kind of 'vaccination' were used both by injection and by inhalation to make allergic patients less reactive. Probably, these attempts have to be seen in the activation of the innate immune system via TOLL-like receptor (TLR) stimulation through bacterial proteins or nucleotides (e.g. CpG, one of the most recent immune response modifying adjuvants) (32).

Table 5 Units for allergen extracts used in the past and present for standardization

W/V	Weight/volume
Noon	1/1000000 g Pollen
PNU	Protein nitrogen unit
HEP	Histamine equivalent prick
IU	International unit
AU	Allergy unit
BAU	Biological allergy unit
BU	Biological unit
IR	Index of reactivity
TU	Therapeutic unit

Mechanisms proposed

Over the century, a multitude of hypothetical concepts was proposed and discussed to explain the effect of ASIT (Table 6), illustrating both the progress in allergological research and of the influence of 'fashion trends' in science (33, 34).

First clinical trials

It was not until the 1950s that the first real clinical trials with allergen-specific immunotherapy were performed, and a great credit goes to William Frankland in England who performed one of the first controlled trials showing that hyposensitization was significantly more effective in higher dose than in a lower dose for treating hay fever (35). Between 1950 and 1980, allergen-specific immunotherapy was used more and more all over the world with different extracts and modalities.

While in the United States the individual allergist was – and often still is – preparing the individual extract for his/her patient from a bulk preparation produced by pharmaceutical companies, in Europe allergen-producing companies were increasingly interested in preparing more purified and better standardized products leading to the preparation of so-called fixed combinations of various allergen mixtures in one final product, which then has to be registered by national authorities.

Allergoids

In trying to improve both efficacy and safety of allergen-specific immunotherapy, the concept of allergoids was born in the 1970s. By chemical modification of allergenic proteins using either formaldehyde or glutaraldehyde, so-called allergoids were prepared, which showed the same amount of immunogenicity, but less 'allergenicity' in the sense of untoward side reactions. While formaldehyde was used to generate allergoids in the United States, (36, 37) the first European formulations were prepared with glutaraldehyde (38–40).

Table 6 Proposed mechanisms of ASIT

Induction of specific 'blocking' IgG antibodies
Antigen blocking
IgE synthesis reducing
IgE receptor blocking (on mast cells and basophils)
Anti-idiotypic antibodies
Mucosal secretion of IgG and IgA antibodies
Reduction in influx of inflammatory cells through
Reduced recruitment
Reduced activation
Reduced 'releasability' (mediators)
Tachyphylaxis
Hapten inhibition
Effect of autacoids (histamine, prostaglandin E)
Induction of T-cell anergy (only 1 signal)
Induction of B-cell tolerance
Switch from Th2 to Th1
Regulatory T cells (Tregs)

ASIT, Allergen-specific immunotherapy.

Allergoids are until today a mainstay in allergen-specific immunotherapy and can be administered in rather high doses after absorption to aluminiumhydroxide (Alum) or other adjuvants. A very recent development is small carbamylated allergoids that are resistant to digestion by proteolytic enzymes and rapidly absorbed via the oral mucosa and thus are used for sublingual immunotherapy of mite and grass allergy (41).

ASIT with adjuvants coupled with allergen

While in the early days allergen extracts were mostly used as aqueous solutions, after the 2nd World War it became clear that to reduce the number of injections a depot- or semi-depot preparation would be better. In analogy to classical vaccines (e.g. tetanus), aluminiumhydroxide (Alum) was used as adjuvant and semi-depot mediator in most allergen preparations.

In the last decades, other modalities were tried, namely tyrosine, calciumphosphate, or monophosphoryl lipid (MPL), which is a derivative of lipopolysaccharide (LPS) derived from Salmonella Minnesota that signals via the Toll-like receptor TLR4 (42). Finally, MPL-coupled allergy vaccines have shown promising results in two recent clinical trials: Grass, tree, or ragweed pollen-allergic patients, who suffered from seasonal allergic rhinitis, responded to a short-course vaccine with four pre-seasonal injections. This novel short-course vaccine reduced rhinoconjunctivitis symptoms and medication use and elevated allergen-specific IgG and IgE upon allergen exposure (43, 44). To prolong allergen-release, the chemically modified MPL-coupled allergen was absorbed to a L-tyrosine depot. Another approach was using polyethylenglycol-coupled allergens (45).

In the 1990s, another new door for ASIT was opened with the identification of new immune response modifiers that can be combined with allergen to modulate the allergic Th2-immune response. Especially CpG-oligonucleotides were shown to induce strong Th1 polarization of immune responses (32, 46). Here, clinical studies in ragweed allergy had shown to decrease the symptoms of nasal inflammation (46, 47).

Insect venom allergy

Apart from pollinosis and airway allergy, also other types of allergic diseases were treated, e.g. insect allergy. In the history of allergen-specific immunotherapy, insect venom anaphylaxis is particularly interesting, since it shows the importance of good allergen extracts in a very illustrative way.

In the past, extracts of the whole body of bees or wasps were used, and there are early papers describing beneficial effects; one of them by Benson and Semenov reads very well and gave the basis to the whole-body extract treatment of insect sting anaphylaxis over decades (48). However, Benson and Semenov had been treating a beekeeper who was anaphylactic, but also suffered from asthma whenever he was taking care of the honey bees. So probably they did something good in injecting relevant allergen for his asthma, but probably not for his anaphylactic episodes due to the venom (48).

Dr. Loveless was studying beekeepers and recognized the natural experiment which these individuals underwent every day as an active immunization. She was able to transfer immunity with beekeeper's serum and thus founded the concept of 'blocking' antibodies (49).

Whole-body extracts of bees and wasps were used over decades to treat insect venom anaphylaxis. Obviously, allergists were satisfied with the effect, and as a matter of fact almost half of the patients seemed to benefit from this type of therapy.

Already in the 1930s, Heinrich Mack, a pharmacist in Illertissen, Southern Germany, who was a hobby bee-keeper was interested in preparing purified bee venom; he did this using electrical stimulation and was able to produce purified bee venom solutions (called 'Forapin') which he wanted to use in rheumatological diseases (50).

This Forapin was also used by early allergists in the 1930s for hyposensitization in bee anaphylaxis with some success, however also with remarkable side-effects and anaphylactic reactions (51). That is why this treatment was not taken up by routine.

It was only in the 1970s in the United States, when the famous double-blinded, placebo-controlled trial was performed by L. Lichtenstein and co-workers in Baltimore, where they compared whole-body extract, bee venom extract, and placebo in bee venom allergic patients. The study had to be discontinued when it became clear that the same amount of patients treated with whole-body extract was reacting anaphylactically as those in the placebo group, while bee venom-treated patients were protected in the sting challenge. Remarkably, it was a percentage of over 40% of patients tolerating the bee sting, although only treated by placebo. This was the same range for the whole-body extract. This classical study already showed the high rate of placebo efficacy in clinical trials in allergy (52).

Guidelines on ASIT

In the second half of the 20th century, in many countries, guidelines were developed by national and regional allergy societies as to how to apply ASIT or hyposensitization in the best possible manner.

A major breakthrough occurred when guidelines on SIT were developed at an international level under the auspices of the World Health Organization (WHO) (2).

Meanwhile national and international guidelines have been improved and have found access to general treatment recommendations, such as of the Allergic Rhinitis and its Impact on Asthma (ARIA) or Global Initiative in Asthma and Respiratory Diseases (GARD) (15, 53).

Modalities of ASIT

There are many modalities, which have been or are used to apply ASIT (Table 7). While the classical method is the subcutaneous injection of allergen extracts (SCIT), since the beginning of the 20th century, other application routes have been tried like oral, nasal, rectal (mucosal tolerance induction) and intradermal scarification (1, 8).

There was a long-lasting debate with partly vehement emotional discussions at international allergy congresses as to whether oral immunotherapy might be effective or not. Many attendants still have in mind the heated discussion between Ferdinand Wortmann and Gunnar Johansson at the congress of the European Academy of Allergology and Clinical Immunology (EAACI) in Vienna in 1980.

It was not until the method of application was refined to the sublingual route when this type of ASIT modality became scientifically accepted after a variety of very well-controlled clinical trials had been performed (SLIT) (15, 54).

There seems to be a cultural impact with regard of the modalities, since the proponents of sublingual immunotherapy in Europe mostly came from Romanic Southern European countries, while the supporters of the classical subcutaneous immunotherapy were found rather in the Nordic Anglo-Saxon region.

Meanwhile, sublingual immunotherapy is not only used with liquid allergen preparations, but also with lyophilized allergen tablets (55). Another new approach is intra lymph node injection of allergen to increase efficacy and reduce the number of injections (56).

Table 7 ASIT modalities (allergen extract, formulation, preparation)

Producer, bulk solution
Allergen extract, formulation, preparation
Aqueous *vs* Semidepot extracts
Allergens *vs* Allergoids
Selection of adjuvant
Route of application (s.c., oral, sublingual, etc.)
When sublingual: Droplets *vs* Tablets
Time of the year to start (pre-, coseasonal)
Duration
Short term *vs* longterm treatment (number of injections)
Regimen of introduction and maintenance
classical
rush
ultrarush
cluster
etc.
Combination with biologics (e.g. anti-IgE)

ASIT, Allergen-specific immunotherapy; s.c., subcutaneous.

Recombinant allergens

In the attempt to better standardize allergen extracts, improvement was achieved by better purification steps, including gel filtration and dialysis, etc. At the end of the 1980s the first production of recombinant allergens using gene technology approaches in vitro was a major milestone in the history of allergology (57, 58).

The first clinical trials with recombinant allergens were carried out with the insect venom anaphylaxis where it was shown that they are equally effective in diagnosis and treatment using phospholipase A 1 preparations (59).

Future of allergen-specific therapy

Over the last 20 years, a large number of new therapeutic concepts have emerged and are currently evaluated or discussed for future application (Table 8). Most of these use chemically or genetically modified proteins or peptides with the goal of reducing IgE-binding and thus anaphylaxis and a booster of allergen-specific IgE, while targeting allergen-specific T cells (for details, see excellent reviews by Valenta et al. and Akdis et al.) (60, 61).

Bet v 1 coupled with a bacterial surface layer protein induced preferential Th1-polarization of the T-cell response, but so far no clinical studies showing safety and efficacy have been provided (62).

Another adjuvant candidate is the TLR2-ligand lipopeptide, which has shown immunoregulatory properties in mouse models of murine immunodeficiency virus, Schistosoma mansoni- and Nippostrongylus brasiliensis infections. In these models of inflammation, TLR2-signaling inducted by lipopeptide led to increased production of IFN-γ and IL-10, while production of the Th2 master-cytokine IL-4 was suppressed (63).

Heat-killed Mycobacterium vaccae is used as an adjuvant in tumor-directed immunotherapies, helping to overcome tumor antigen-specific anergy (64). However, also an allergy preventing Th1 effect has been observed in a mouse model of allergy, but this approach has not been led to human studies (65).

Finally, a fusion of Der p 1 peptide to virus-like particles was shown to be safe and highly immunogenic in healthy adults (66). However, future studies need to address whether this approach can downregulate allergic Th2-sensitization, or at least be used to prevent the spread of the sensitization status to high-risk allergens that currently do not elicit allergic symptoms in an individual.

Table 8 Future of allergen-specific immunotherapy (ASIT)

More pure and standardized extracts
Component-resolved ASIT
Allergen mutants (isoallergens)
Allergen peptides
Chimeras
Fusion proteins
Naked DNA vaccine
Combination with biologics
Application routes and galenics
Adjuvants
Combination with viral vaccines

Cytokine inhibitors targeting general T-cell activation (Daclizumab) and effector functions [TNF-α or Th2-cytokines (IL-4, IL-5)] specifically might represent 'reverse-adjuvants' or 'tolerogens' that could enhance the effect of conventional SIT (67–70). However, the effectiveness of this approach is currently mere speculation, but owing to the availability of respective monoclonal antibodies with a good safety profile, clinical studies could be carried out.

Recombinant wild-type allergens

Another very promising approach for ASIT comprises recombinant wild-type allergens, which resemble the complete immunological and structural properties of the natural allergen. These in vitro-generated molecules bear the advantage that they can be included in therapeutic formulations in defined quantity and tailored to the patient's sensitization status. In contrast, currently used allergen extracts have the drawback of inconsistent composition, and in some cases, owing to chemico-physical properties, a commercially available extract does not contain the relevant allergen for a patients individual sensitization (61). Another drawback of the current ASIT using allergen extracts is the presence of proteins that can induce neo-sensitization during the course of treatment. Moreover, allergen extracts can also contain bioactive substances that have proinflammatory and Th2-promoting capacities (71–73). The use of pure recombinant wild-type allergens can help to overcome these potential risks. However, these proteins still contain the IgE-binding epitopes of their naturally occurring counterparts, which makes the proteins an interesting diagnostic tool. To overcome the IgE-mediated side-effects, a recombinant hypoallergenic allergen derivative has been created recently (74). Clinical trials with recombinant wild-type allergens using a mixture of grass pollen allergens or a sole birch pollen allergen (Bet v 1) were successful and proved the principle of recombinant allergens as being very promising (75, 76).

Using recombinant or synthetically allergens will offer several advantages over the use of allergen extracts:

Defined molecules can be produced with consistent quality and unlimited amounts. Recombinant approaches using random- or site-directed mutagenesis, deletions without IgE epitopes, recombinant fragments, or the reassembly of molecules that have in part been recombined, can be used in the form of proteins or peptides with reduced allergenicity, i.e. less IgE side-effects (61, 77).

T cell-based epitope vaccines

In the 1990s, the first T cell-based epitope vaccines were evaluated. This approach uses allergen-derived peptides that contain T-cell epitopes without IgE-binding capacities (78–80). Although CD4 T-cell anergy, an allergen-specific Th1-shift and CD4 T-cells with regulatory activity can be induced by these peptides, only few studies have shown significant improvements on allergic symptoms (59, 79, 81). As attempted, no IgE-mediated side-effects have occurred, but late phase reactions that were most likely T-cell-mediated, have been observed in many studies (78, 79, 82). However, because IgE-mediated symptoms are not crucially affected by T-cell-based epitope vaccines, transition of this concept into clinical practice has not been achieved. A potential pitfall is the fact that initial B-cell sensitization requires T-cell help, whereas subsequent booster reactions can take place independently from T-cells, so that a sole T-cell-mediated treatment of atopic diseases is inefficient (83, 84).

Hypoallergenic isoallergens

The risk of IgE-mediated anaphylaxis despite regulation of T-cell-responses could be overcome by development of so-called hypoallergens (recombinant or chemically induced hypoallergenic allergen derivatives) (85, 86). These elegantly designed molecules possess T-cell epitopes plus IgG epitopes that combine the advantage of inducing allergen-blocking IgG and regulating allergen-specific T-cell responses (87, 88). A number of successful trials with birch allergic patients (Bet v 1) have been conducted over the last years, and a first drug based on recombinant birch pollen allergen is expected to enter the market for routine ASIT (61, 89).

Hybrid molecules

To facilitate the production of an allergy vaccine, the construction of hybrid molecules is a way to reduce the complexity of future drugs. In addition, hybrid molecules show higher immunogenicity, which should enhance their therapeutic potential (83, 90). However, to reduce the danger of IgE-mediated anaphylaxis, the principles of hypoallergenic allergen derivatives with exclusion of IgE epitopes have been applied in a recent study (74). Because of the feasibility of producing hypoallergenic hybrid molecules, we await further promising studies using this approach with subsequent development of improved routine ASIT.

Combination of anti-IgE plus conventional ASIT

As allergen SIT carries the inherit risk of severe anaphylactic reactions, a combination of conventional ASIT with an anti-IgE antibody (omalizumab) was evaluated. Interestingly, the combination therapy leads to a further reduction in allergic symptoms in verum-treated patients without increase in

severe side-effects (91). Moreover, in rush ASIT, the risk of anaphylaxis was diminished compared to ASIT only. At least in part, the mechanism could be revealed as the blocking of allergen-binding to IgE molecules (92).

IgE-reactive haptens and mimotopes

In contrast to the above-mentioned recombinant allergens, IgE-reactive haptens are far from use in clinical settings. However, the concept is intriguing, as IgE-reactive haptens provide one IgE-binding site, which allows them to block IgE on mast cells and basophils (93, 94).

Allergen-mimotopes compete with the cognate ligand of IgE molecules and thereby interfere with IgE crosslinking, similar to IgE-reactive haptens. Mimotopes with capacity to interfere with grass pollen-specific IgE have been isolated from synthetic peptide-libraries already in 2001. Clinical studies showing superior efficacy of these concepts are still to be published (95, 96).

DNA vaccination

In theory, the production of allergen-encoding DNA should be cheaper and the product more stable than the production of proteins. Thus, several groups have studied mouse models of genetic vaccination using naked DNA, which should be able to deliver the corresponding antigens of a patient's sensitization status. However, many unresolved issues preclude genetic vaccination from becoming a routine treatment for humans in the near future (61): (i) identification of suitable vectors, (ii) mode of application, and (iii) application site.

A major danger of genetic vaccination is persistence of an allergen that causes side-effects and the development of immune reactions against vector sequences. Therefore, rapidly degradable nucleic acids that can deliver hypoallergens are under development (97, 98).

Gene therapy

An innovative, but yet purely experimental treatment option is gene therapy, in which allergens could be transfected into cells and sites where immune tolerance is induced (mainly bone marrow, thymus, spleen, lymph nodes, or liver). However, to date only murine models address this option in a prophylactic setting using grass pollen (Phleum p 5) antigen (98–100). Given the development of safe vectors and generation of suitable hypoallergens, gene therapy might be suitable especially for prophylactic treatment of patients at risk.

Conclusion and outlook

During the last 100 years, we have gained tremendous insight into the pathogenesis of atopic diseases and the mechanisms that are triggered by the different modalities of allergen-specific immunotherapy. After initially being an experimental approach with frequent and severe side-effects, ASIT has become the gold standard for the causative treatment for IgE-mediated allergic diseases for a large variety of allergens. One may curiously await the new developments, which will further enhance our understanding of allergy mechanisms and improve ASIT for the next generations of patients and physicians.

References

1. Ring J. Allergy in Practice. Berlin Heidelberg New York: Springer, 2005.
2. Bousquet J, Lockey R, Malling H-J, the WHO panel members. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol* 1998;**102**:558–562.
3. de Weck AL, Ring J. CIA, Collegium Internationale Allergologicum: History and Aims of a Special International Community Devoted to Allergy Research, 1954–1996, 2nd edn. Munich: Urban Vogel, 2006.
4. Noon L. Prophylactic inoculations against hay fever. *Lancet* 1911;**1**:1572–1573.
5. Simons FER (ed.). Ancestors of allergy. *Global Medical Communications*, New York, 1994, pp. 114–117.
6. Cooke RA. Studies in Specific Hypersensitiveness: IX. On the Phenomenon of Hyposensitization (the Clinically Lessened Sensitiveness of Allergy). *J Immunol* 1922;**7**: 219–242.
7. Pichler WJ. Specific and nonspecific (anti-IgE) immunotherapy of allergic diseases. *Ther Umsch* 2001;**58**:329–336.
8. Schadewaldt H. Geschichte der Allergie, Band 1–4. 1 ed. Muenchen-Deisenhofen: Dustri-Verlag, 1979–1983.
9. Jenner E. An inquiry into the causes and effects of the variolae vaccinae. A disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of the cow pox. Low, London, 1798.
10. Giel S. Die Schutpockenimpfung in Bayern, vom Anbeginn ihrer Entstehung und gesetzlichen Einführung bis auf gegenwärtige Zeit, dann mit besonderer Beachtung derselben in anderen Staaten. *Bibliotheca Regia Monacensis*, Munich, 1830, p. 105.
11. Hahnemann S. Die chronischen Krankheiten, ihre eigentümliche Natur und ihre homöopathische Heilung *Arnoldische Buchhandlung, Dresden und Leipzig* 1828.
12. Pipet A, Botturi K, Pinot D, Vervloet D, Magnan A. Allergen-specific immunotherapy in allergic rhinitis and asthma. Mechanisms and proof of efficacy. *Respir Med* 2009;**103**: 800–812.
13. Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for seasonal allergic rhinitis. *Cochrane Database Syst Rev* 2007;**1**:CD001936.
14. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2005;**60**:4–12.
15. Bousquet J, Dahl R, Khaltaev N. Global alliance against chronic respiratory diseases. *Allergy* 2007;**62**:216–223.
16. Dunbar WP. Zur Ursache und spec. Heil. des Heufiebers. *Dtsch Med Wochenschr* 1903;**9**:24–28.
17. Dunbar WP. Zur Frage betreffend der Ätiologie und specifischen Heilung des Heufiebers. *Berl Klin Wochenschrift* 1903;**40**: 569.
18. Wolff-Eisner A. Das Heufieber: sein Wesen und seine Behandlung. München: Lehmann, 1906.
19. Besredka A. Comment empecher l'anaphylaxie? *C R Soc Biol* 1907;**59**: 1053.

20. Besredka A. Du mecanisme de l'anaphylaxie vis-a-visde serum de cheval *C R Soc Biol* 1907;**59**:294–296.
21. Stead RH, Tomioka M, Quinonez G, Simon GT, Felten SY, Bienenstock J. Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc Natl Acad Sci USA* 1987;**84**:2975–2979.
22. Curtis HH. The immunizing cure of hay fever. *NY Med J* 1900;**77**:16–18.
23. Rosenau MJ, Anderson JF. A new toxic Action of Horse Serum. *J Med Res* 1906; **15**:179–208.
24. Pirquet Cv, Schick B. Die Serumkrankheit. *Leipzig-Wien* 1905.
25. Scheppegrell W. The Immunizing Treatment of Hay Fever. *NY Med J* 1909;**90**:1099.
26. Freeman J. Further observations on the treatment of hay fever by hypodermic inoculations of pollen vaccine. *Lancet* 1911;**178**: 814–817.
27. Cooke RA. The treatment of hay fever by active immunization. *Laryngoscope* 1914;**25**: 108–112.
28. Pasteur Vallery-Radot P, Blamoutier P. La cutièreaction dans l'asthme, la coryza spasmodique, le rhume de foins, la migraine, l'urticaire et l'èczèma. *Presse Mèd* 1925; **33**:385.
29. Lambert A, Bouin P, Ancel AP. Sur la skeptophylaxis. *CR Soc Biol* 1911;**71**:350.
30. Fell N, Rodney G, Marshall DE. Histamine-protein complexes: synthesis and immunologic investigation: I. histamine-azoprotein. *J Immunol* 1943;**47**:237–249.
31. Cohen MB, Friedman HJ. Antibodies to histamine induced in human beings by histamine conjugates. *J Allergy* 1943;**14**:195–202.
32. Sato Y, Roman M, Tighe H, Lee D, Corr M, Minh-Duc N et al. Immunostimulatory DNA sequences necessary for effective intradermal gene immunization. *Science* 1996; **273**:352–354.
33. Jutel M, Akdis M, Blaser K, Akdis CA. Mechanisms of allergen specific immunotherapy–T-cell tolerance and more. *Allergy* 2006;**61**:796–807.
34. Jutel M, Akdis M, Budak F, Aebischer-Casaulta C, Wrzyszcz M, Blaser K et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol* 2003;**33**:1205–1214.
35. Frankland AW. High and low dosage pollen extract treatment in summer hay fever and asthma. *Acta Allergol* 1955;**9**:183–187.
36. Norman PS, Lichtenstein LM, Marsh DG. Studies on allergoids from naturally occurring allergens. IV. Efficacy and safety of long-term allergoid treatment of ragweed hay fever. *J Allergy Clin Immunol* 1981;**68**: 460–470.
37. Marsh DG, Norman PS, Roebber M, Lichtenstein LM. Studies on allergoids from naturally occurring allergens. III. Preparation of ragweed pollen allergoids by aldehyde modification in two steps. *J Allergy Clin Immunol* 1981;**68**:449–459.
38. Johansson SGO, Deuschl H, Zetterstrom O. Use of glutaraldehyde-modified timothy grass pollen extract in nasal hyposensitisation treatment of hay fever. *Int Arch Allergy Appl Immunol* 1979;**60**:447–460.
39. Foucard T, Johansson SGO. Immunological studies in vitro and in vivo of children with pollenosis given immunotherapy with an aqueous and a glutaraldehyde-treated tyrosine-absorbed grass pollen extract. *Clin Allergy* 1976;**6**:429–439.
40. Johansson SGO, Miller AC, Mullan N, Overell BG, Tees EC, Wheeler A. Glutaraldehyde-pollen-tyrosine: clinical and immunological studies. *Clin Allergy* 1974;**4**:255–263.
41. Mösges R, Ritter B, Kayoko G, Allekotte S. Carbamylated monomeric allergoids as a therapeutic option for sublingual immunotherapy of dust mite- and grass pollen-induced allergic rhinoconjunctivitis: a systematic review of published trials with a meta-analysis of treatment using Lais tablets. *Acta Dermatovenerol Alp Panonica Adriat* 2010;**19**:3–10.
42. Johnson AG, Tomai M, Solem L, Beck L, Ribi E. Characterization of a nontoxic mono phosphoryl lipid. *Rev Infect Dis* 1987;**9**:512–516.
43. Patel P, Salapatek A. Pollinex Quattro: a novel and well-tolerated, ultra short-course allergy vaccine. *Expert Rev Vaccines* 2006; **5**:617–629.
44. Puggioni F, Durham SR, Francis JN. Monophosphoryl lipid A (MPL) promotes allergeninduced immune deviation in favour of Th1 responses. *Allergy* 2006;**60**:678–684.
45. Lee WY, Sehon AH. Abrogation of reaginic antibodies with modified allergens. *Nature* 1977;**267**:618–619.
46. Tulic MK, Fiset PO, Christodoulopoulos P, Vaillancourt P, Desrosiers M, Lavigne F et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. *J Allergy Clin Immunol* 2004;**113**: 235–241.
47. Creticos PS, Schroeder JT, Hamilton RG, Balcer-Whaley SL, Khattignavong AP, Lindblad R et al. Immunotherapy with a ragweed-Toll-like receptor 9 agonist vaccine for allergic rhinitis *N Engl J Med* 2006;**355**: 1445–1455.
48. Benson RL, Semenov H. Allergy in its relation to bee sting. *J Allergy* 1930;**1**:105.
49. Loveless MH, Cann JR. Distribution of allergic and blocking activity in human serum proteins fractionated by electrophoresis convection. *Science* 1953;**117**:105–108.
50. Hahn G, Ostermayer H. Ueber das Bienengift. *Berichte der Deutschen Chemischen Gesellschaft* 1936;**11**:2407.
51. Lotter G. Sensibilisierung für Bienengift durch Typhus-Antitoxin und Desensibilisierung mit Forapin. *Münch Med Wochenschr* 1939; **83**:330–331.
52. Hunt KJ, Valentine MD, Sobotka AK, Benton AW, Amodio FJ, Lichtenstein LM. A controlled trial of immunotherapy in insect hypersensitivity. *N Engl J Med* 1978;**299**: 157–161.
53. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008;**63**(Suppl 86):8–160.
54. Compalati E, Canonica GW, Passalacqua G, Baena-Cagnani CE. Considerations about the evaluation of the SLIT meta-analyses. *J Allergy Clin Immunol* 2010;**125**: 509; author reply 509-10.
55. Durham SR, Emminger W, Kapp A, Colombo G, de Monchy JG, Rak S et al. Long-term clinical efficacy in grass pollen-induced rhinoconjunctivitis after treatment with SQ-standardized grass allergy immunotherapy tablet. *J Allergy Clin Immunol* 2010; **125**:131-8 e1-7.
56. Senti G, Prinz Vavricka BM, Erdmann I, Diaz MI, Markus R, McCormack SJ et al. Intralymphatic allergen administration renders specific immunotherapy faster and safer: a randomized controlled trial. *Proc Natl Acad Sci USA* 2008;**105**:17908–17912.
57. Tovey ER, Johnson MC, Roche AL, Cobon GS, Baldo BA. Cloning and sequencing of a cDNA expressing a recombinant house dust mite protein that binds human IgE and corresponds to an important low molecular weight allergen. *J Exp Med* 1989;**170**: 1457–1462.
58. Breiteneder H, Pettenburger K, Bito A, Valenta R, Kraft D, Rumpold H et al. The gene coding for the major birch pollen allergen Betv1, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989;**8**:1935–1938.
59. Müller U, Akdis CA, Fricker M, Akdis M, Blesken T, Bettens F et al. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998; **101**:747–754.
60. Akdis M, Akdis C. Therapeutic manipulation of immune tolerance in allergic disease. *Nat Rev Drug Discov* 2009;**8**:645–660.
61. Valenta R, Ferreira F, Focke-Tejkl M, Linhart B, Niederberger V, Swoboda I et al. From allergen genes to allergy vaccines. *Annu Rev Immunol* 2010;**28**:211–241.

62. Bohle B, Breitwieser A, Zwolfer B, Jahn-Schmid B, Sara M, Sleytr UB et al. A novel approach to specific allergy treatment: the recombinant fusion protein of a bacterial cell surface (S-layer) protein and the major birch pollen allergen Bet v 1 (rSbsC-Bet v 1) combines reduced allergenicity with immunomodulating capacity. *J Immunol* 2004; **172**:6642–6648.
63. Akdis CA, Kussebi F, Pulendran B, Akdis M, Lauener R, Schmidt-Weber CB et al. Inhibition of T helper 2-type responses, IgE production and eosinophilia by synthetic lipopeptides. *Eur J Immunol* 2003;**33**:2717–2726.
64. Grange JM, Bottasso O, Stanford CA, Stanford JL. The use of mycobacterial adjuvant-based agents for immunotherapy of cancer. *Vaccine* 2008;**26**:4984–4990.
65. Zuany-Amorim C, Manlius C, Trifilieff A, Brunet LR, Rook G, Bowen G et al. Long-term protective and antigen-specific effect of heat-killed Mycobacterium vaccae in a murine model of allergic pulmonary inflammation. *J Immunol* 2002;**169**:1492–1499.
66. Kündig TM, Senti G, Schnetzler G, Wolf C, Prinz Vavricka BM, Fulurija A et al. Der p 1 peptide on virus-like particles is safe and highly immunogenic in healthy adults. *J Allergy Clin Immunol* 2006;**117**:1470–1476.
67. Nelson HS, Busse WW, Israel E, Baker JW, Charous BL, Kim KT et al. Daclizumab improves asthma control in patients with refractory asthma. *J Allergy Clin Immunol* 2005;**115**:134.
68. Morjaria JB, Chauhan HJ, Babu KS, Polosa R, Davies DE, Holgate ST. The role of a soluble TNFalpha receptor fusion protein (etanercept) in corticosteroid refractory asthma: a double blind, randomised, placebo controlled trial. *Thorax* 2008;**63**:584–591.
69. Flood-Page P, Menzies-Gow A, Phipps S, Ying S, Wangoo A, Ludwig MS et al. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003;**112**:1029–1036.
70. Borish LC, Nelson HS, Lanz MJ, Claussen L, Whitmore JB, Agosti JM et al. Interleukin-4 receptor in moderate atopic asthma. A phase I/II randomized, placebocontrolled trial. *Am J Respir Crit Care Med* 1999; **160**:1816–1823.
71. Gutermuth J, Bewersdorff M, Traidl-Hoffmann C, Ring J, Mueller MJ, Behrendt H et al. Immunomodulatory effects of aqueous birch pollen extracts and phytoprostanes on primary immune responses in vivo. *J Allergy Clin Immunol* 2007;**120**:293–299.
72. Traidl-Hoffmann C, Mariani V, Hochrein H, Karg K, Wagner H, Ring J et al. Pollen-associated phytoprostanes inhibit dendritic cell interleukin-12 production and augment T helper type 2 cell polarization. *J Exp Med* 2005;**201**:627–636.
73. Focke M, Marth K, Flicker S, Valenta R. Heterogeneity of commercial timothy grass pollen extracts. *Clin Exp Allergy* 2008; **38**:1400–1408.
74. Linhart B, Mothes-Luksch N, Vrtala S, Kneidinger M, Valent P, Valenta R. A hypoallergenic hybrid molecule with increased immunogenicity consisting of derivatives of the major grass pollen allergens, Phl p 2 and Phl p 6. *Biol Chem* 2008;**389**:925–933.
75. Jutel M, Jaeger L, Suck R, Meyer H, Fiebig H, Cromwell O. Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol* 2005;**116**:608–613.
76. Pauli G, Larsen TH, Rak S, Horak F, Pastorello E, Valenta R et al. Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis. *J Allergy Clin Immunol* 2008;**122**:951–960.
77. Valenta R, Niederberger V. Recombinant allergens for immunotherapy. *J Allergy Clin Immunol* 2007;**119**:826–830.
78. Haselden BM, Kay AB, Larché M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 1999;**189**:1885–1894.
79. Norman PS, Ohman JL Jr, Long AA, Creticos PS, Gefter MA, Shaked Z et al. Treatment of cat allergy with T-cell reactive peptides. *Am J Respir Crit Care Med* 1996;**154**:1623–1628.
80. Verhoef A, Alexander C, Kay AB, Larche M. T cell epitope immunotherapy induces a CD4+ T cell population with regulatory activity. *PLoS Med* 2005;**2**:e78.
81. Oldfield WL, Larche M, Kay AB. Effect of T-cell peptides derived from Fel d 1 on allergic reactions and cytokine production in patients sensitive to cats: a randomised controlled trial. *Lancet* 2002;**360**:47–53.
82. Haselden BM, Larche M, Meng Q, Shirley K, Dworski R, Kaplan AP et al. Late asthmatic reactions provoked by intradermal injection of T-cell peptide epitopes are not associated with bronchial mucosal infiltration of eosinophils or T(H)2-type cells or with elevated concentrations of histamine or eicosanoids in bronchoalveolar fluid. *J Allergy Clin Immunol* 2001;**108**: 394–401.
83. Linhart B, Hartl A, Jahn-Schmid B, Verdino P, Keller W, Krauth MT et al. A hybrid molecule resembling the epitope spectrum of grass pollen for allergy vaccination. *J Allergy Clin Immunol* 2005;**115**:1010–1016.
84. Linhart B, Bigenzahn S, Hartl A, Lupinek C, Thalhamer J, Valenta R et al. Costimulation blockade inhibits allergic sensitization but does not affect established allergy in a murine model of grass pollen allergy. *J Immunol* 2007;**178**:3924–3931.
85. Valenta R. The future of antigen-specific immunotherapy of allergy. *Nat Rev Immunol* 2002;**2**:446–453.
86. Kahlert H, Suck R, Weber B, Nandy A, Wald M, Keller W et al. Characterization of a hypoallergenic recombinant Bet v 1 variant as a candidate for allergen-specific immunotherapy. *Int Arch Allergy Immunol* 2008;**145**:193–206.
87. Niederberger V, Horak F, Vrtala S, Spitzauer S, Krauth MT, Valent P et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci USA* 2004;**101**(Suppl 2): 14677–14682.
88. Vrtala S, Focke-Tejkl M, Swoboda I, Kraft D, Valenta R. Strategies for converting allergens into hypoallergenic vaccine candidates. *Methods* 2004;**32**:313–320.
89. Rak S. Clinical results with a hypoallergenic recombinant birch pollen allergen derivative. *Presented at 27th Congr., EAACI 2009, Warsaw, June 6–10* 2009.
90. King TP, Jim SY, Monsalve RI, Kagey-Sobotka A, Lichtenstein LM, Spangfort MD. Recombinant allergens with reduced allergenicity but retaining immunogenicity of the natural allergens: hybrids of yellow jacket and paper wasp venom allergen antigen 5s. *J Immunol* 2001;**166**:6057–6065.
91. Casale TB, Busse WW, Kline JN, Ballas ZK, Moss MH, Townley RG et al. Omalizumab pretreatment decreases acute reactions after rush immunotherapy for ragweed-induced seasonal allergic rhinitis. *J Allergy Clin Immunol* 2006;**117**: 134–140.
92. Klunker S, Saggar LR, Seyfert-Margolis V, Asare AL, Casale TB, Durham SR et al. Combination treatment with omalizumab and rush immunotherapy for ragweedin-duced allergic rhinitis: Inhibition of IgE-facilitated allergen binding. *J Allergy Clin Immunol* 2007;**120**:688–695.
93. Ball T, Vrtala S, Sperr WR, Valent P, Susani M, Kraft D et al. Isolation of an immunodominant IgE hapten from an epitope expression cDNA library. Dissection of the allergic effector reaction. *J Biol Chem* 1994;**269**:28323–28328.
94. Segal DM, Taurog JD, Metzger H. Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. *Proc Natl Acad Sci USA* 1977;**74**:2993–2997.
95. Ganglberger E, Sponer B, Schöll I, Wiedermann U, Baumann S, Hafner C et al. Monovalent fusion proteins of IgE mimo-

topes are safe for therapy of type I allergy. *FASEB J* 2001;**15**:2524–2526.
96. Suphioglu C, Schäppi G, Kenrick J, Levy D, Davies JM, O'Hehir R. A novel grass pollen allergen mimotope identified by phage display peptide library inhibits allergen-human IgE antibody interaction. *FEBS Lett* 2001;**502**:46–52.
97. Bauer R, Scheiblhofer S, Kern K, Gruber C, Stepanoska T, Thalhamer T et al. Generation of hypoallergenic DNA vaccines by forced ubiquitination: preventive and therapeutic effects in a mouse model of allergy. *J Allergy Clin Immunol* 2006; **118**:269–276.
98. Hochreiter R, Stepanoska T, Ferreira F, Valenta R, Vrtala S, Thalhamer J et al. Prevention of allergen-specific IgE production and suppression of an established Th2-type response by immunization with DNA encoding hypoallergenic allergen derivatives of Bet v 1, the major birch-pollen allergen. *Eur J Immunol* 2003; **33**:1667–1676.
99. Steptoe RJ, Ritchie JM, Harrison LC. Transfer of hematopoietic stem cells encoding autoantigen prevents autoimmune diabetes. *J Clin Invest* 2003;**111**:1357–1363.
100. Baranyi U, Linhart B, Pilat N, Gattringer M, Bagley J, Muehlbacher F et al. Tolerization of a type I allergic immune response through transplantation of genetically modified hematopoietic stem cells. *J Immunol* 2008;**180**:8168–8175.