

Conference Scene



Novelties in immunotherapy

European Academy of Allergology and Clinical Immunology – World Allergy Organization
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The only method aiming to permanently cure allergic disorders is allergen immunotherapy. Over the last 20 years there has been great progress in understanding the mechanisms that govern allergen immunotherapy in order to meet three basic prerequisites: safety, effectiveness and compliance. In the present summary report from the European Academy of Allergology and Clinical Immunology–World Allergy Organization Congress held last June in Milan, we review key points concerning the main axes as diagnosis, novel modalities, routes and protocols, as well as two important immunotherapy fields: food and insect venom allergy.

‘Allergy: a global health challenge’ was the theme of the European Academy of Allergology and Clinical Immunology – World Allergy Organization World Allergy and Asthma Congress that was hosted in Milan from 22–26 June 2013. Thousands of scientists from all over the world attended the Congress and presented their work and experience on every aspect of the field of allergy and clinical immunology. Immunotherapy was one of the key subjects covered by numerous lectures, symposiums, oral and poster abstract sessions.

Diagnosis

One of the most important elements of allergen immunotherapy (AIT) is that it is specific (i.e., it is aimed at specific allergens that cause the allergic symptoms), no matter if these are the result of exposure to aeroallergens, food or insect venom allergens. Therefore, at the core of effective AIT stands the correct diagnosis of the relevant allergen that causes the symptoms. Until now, history-taking and skin prick tests were the two basic means of attributing symptoms to specific allergens. These two steps are indispensable, but in many cases are not accurate enough. Patients from areas with overlapping flowering periods (e.g., Mediterranean basin), polysensitized patients (i.e., patients with multiple sensitizations) or patients with unclear symptoms are very difficult to deal with. In addition, the presence of panallergens (i.e., allergen components present in pollen of more than one species) results in false-positive skin prick testing. Molecular/component resolved diagnostics (CRD) emerges as an essential third step in the management of these patients since

it can clarify reactivities against species-specific components (i.e., genuine sensitization) compared with cross-reacting components (i.e., panallergens) [1]. Paolo Matricardi and Giovanni Melioli justified that today CRD increases the relative clinical impact of AIT through both prescribing AIT to patients previously considered poor candidates and by electing more specific extracts. In the short term, the molecular profile of each patient will allow allergists to tailor AIT to personal needs [2], while in the future early detection of specific components will probably allow earlier (even preclinical) intervention [3]. As Walter Canonica presented, these are depicted in the upcoming revision of the World Allergy Organization position paper on sublingual AIT (SLIT). In addition, Ignacio Ansotegui demonstrated that sensitization to specific components may predict the risk of reaction to AIT [4]. Many companies presented their approach to multiplex diagnosis (i.e., the simultaneous determination of different antibodies to multiple allergens in single-assay format). However, there are still some unmet requirements of CRD, however, such as important allergens not being available for routine testing, the clinical relevance of many being unknown and regional differences existing. The increased cost of CRD seems to be justified [5]. On the other hand, since AIT is still performed with traditional extracts and the standardization process allows the quantification of only one component, we may have reached a point where CRD is more advanced for AIT available today. Ongoing efforts for accurate identification and measurement of components in the extracts were presented (e.g., mass

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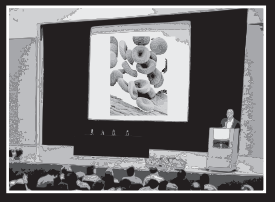
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spectrometry, HPLC, specific monoclonal antibody-based ELISA). However, the full component characterization of the extracts is a challenge for AIT companies in the future.

Novel modalities

Another important challenge is to produce extracts with high immunogenicity, while keeping allergenicity and, subsequently, risk of reactions low. Marianne van Hage analyzed the use of immunomodulators in AIT. These approaches involve combining allergens with immunomodulatory components. Toll-like receptors are an essential component of innate immunity and their activation using Toll-like receptor-4 agonist monophosphoryl-lipid A has been demonstrated to skew immune responses towards the Th1 direction and improve clinical outcomes [6,7]. Analogous results have been achieved with the addition of immunostimulatory DNA sequences (CpG). Type-A CpG seems more potent at eliciting an increase in IFN- γ levels, but also demonstrates instability that resolves upon association with virus-like particles. Coupling virus-like particles directly to allergen has also been proved to induce clinical and immunological responses [8]. In a recent study by Marth *et al.*, the construction and characterization of a HBV surface protein carrier-based peptide vaccine for birch pollen was reported. This vaccine led to a reduction of allergenic activity and CD23-facilitated allergen presentation, and enhanced blocking antibody responses towards IgE epitopes [9]. In murine models of cat allergy, covalent coupling of vitamin D3 to the major cat allergen Fel d1 [10] and adding carbohydrate-based particles [11] resulted in enhanced AIT results. Finally, the utilization of polymeric nanoparticles induces enhanced immunological responses and/or facilitates gradual allergen release. Data from poster sessions suggest that coating microparticles with neuraminidase is a beneficial immunomodulatory approach for oral immunotherapy in food allergy. In the same context, the development of synthetic pathogen-like plasmid DNA/polyethylenimine PEIm nanoparticles expressing ovalbumin induced favorable immunological responses in murine models.

Mark Larché commented on the use of peptides for AIT. Extracts used today have various limitations; cross-linking of specific IgE receptors on mast cells/basophils and therefore dose limitation and adverse reactions is probably the most important. By contrast, peptides consist of immunodominant T-cell epitopes that have reduced secondary/tertiary structure, which does not permit specific IgE receptor-binding and are chemically defined and standardized. Current evidence suggests that intradermal delivery of specific T-cell epitopes leads to the induction of Tregs, which results in an amelioration of the response to antigens. Peptide formulations have been used in patients with honeybee venom and cat allergies, and recently with pollen and mould allergies, showing encouraging results [12]. Intramolecular tolerance (linked epitope suppression) involves the expansion of tolerance to peptides other than those used in treatment, a mechanism very important for successful AIT. In a recent study, Patel *et al.* demonstrated the persistence of the favorable effect of a set of synthetic T-cell epitopes derived from Fel d1 (cat-peptide antigen desensitization [Cat-PAD]) 1 year after therapy. Four administrations of 6 nmol of Cat-PAD 4 weeks apart resulted in improved rhinoconjunctivitis symptom score compared with eight administrations of 3 nmol 2 weeks apart and with the placebo group [13]. The favorable tolerability profile of Cat-PAD was further supported during poster discussion sessions.

Novel routes

Thomas Kündig focused on the rationale and results of novel routes of allergen delivery, particularly intralymphatic (ILIT) and epicutaneous (EPIT) immunotherapy. By injecting allergen directly into lymph nodes, allergen delivery is increased by approximately 100-times compared with other routes. Thus, using a much smaller allergen dose, injection-related adverse effects are reduced and greater immunogenicity is achieved, which is further enhanced when modified immunotherapeutic agents are used, such as adjuvants, DNA, RNA, proteins, oligopeptides or whole cells. Clinical trials have demonstrated promising results on the efficacy and safety of ILIT for the

treatment of birch and grass pollen allergy, honey bee venom hypersensitivity and cat dander allergy.

Senti *et al.* compared ILIT with modular antigen transporter (MAT)–Fel d1 with placebo in allergic patients. Following three intralymphatic injections within 2 months, patients in the MAT–Fel d1 group had an increased nasal tolerance, stimulated Treg responses and increased cat dander-specific IgG4 levels. MAT–Fel d1 was produced by fusing recombinant major cat dander allergen Fel d1 to the HIV-derived translocation sequence in order to bring the allergen into the cytoplasm and to a truncated human invariant chain, generating a MAT vaccine (MAT–Fel d1). Fusion with a truncated human invariant chain aims at evading phagosomal uptake and degradation by targeting the MHC class II molecules in the endoplasmic reticulum [14]. Hylander *et al.* applied the same ILIT protocol with birch or grass pollen. The following pollen season, a clear symptom improvement over baseline was observed in the active group compared with the placebo group. Moreover, nasal symptoms and local inflammatory response (leukocytes, epithelial cells and IL-8 levels) upon intranasal challenge were lower in the active group. ILIT elicited no severe adverse events [15].

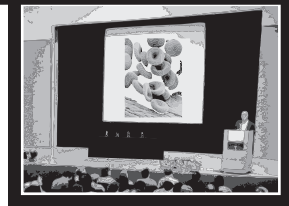
EPIT has the potential to be a safe and efficacious route for AIT due to the skin's unique immunological functions and structure. The epidermis, apart from outer layer keratinocytes, contains antigen-presenting Langerhans cells (LCs) and a potential intervention that targets them can be utilized in favor of immune tolerance induction. The main challenge in EPIT is the disruption of the stratum corneum barrier, important not only to enhance antigen penetration, but also to activate keratinocytes that interact with LCs and secrete cytokines. The scarification technique has now been replaced with novel methods, such as tape stripping (or other abrasive methods), utilizing microneedle arrays with a defined length (aiming at LCs) or extensive skin hydration. Recent clinical trials of EPIT have demonstrated efficacy and a good safety profile, while posters discussed during the Congress suggest a potential future role of EPIT in food allergy. Senti *et al.* demonstrated a dose-dependent improvement

of rhinoconjunctivitis symptoms after six patch applications of grass pollen allergen. Adverse effects were more common as allergen concentration increased and were predominantly local reactions (pruritus, erythema, wheal or eczema). A total of 11 systemic (not severe) reactions were also recorded [16].

Protocols

Patient compliance is of outmost importance for successful AIT. Conventional updosing protocols are time-wasting and in many settings (e.g., patients from rural areas) cannot be implemented. Cluster, rush and ultra-rush protocols for AIT against inhalant allergens last 1 to several days, and are therefore very patient-friendly. Numerous posters during the Congress dealt with the safety of these protocols and assert equal safety to conventional ones, either using whole-allergen extracts or modified allergoids. Arnon Goldberg demonstrated that rush protocols may replace conventional ones in the case of venom immunotherapy; this will probably apply for inhalant allergy as well in the near future.

Similarly, the long duration of maintenance treatment (3–5 years according to latest guidelines) is also a factor of poor compliance. This is addressed by pre-seasonal-only or coseasonal protocols; that is, protocols that last a few months compared with a whole year. Giovanni Pajno demonstrated that in subcutaneous AIT, monthly, year-long intervals are important in order to achieve long-term effects. For SLIT, the continuous regimens seem to perform better during the first year compared with coseasonal ones, whereas in the subsequent years, the two regimens are nearly equivalent [17]. Preseasonal SLIT protocols need to be provided at least 8 weeks before the start of the season in order to be fully effective [18]. Of interest, two posters showed that intraseasonal initiation of AIT (either subcutaneous or sublingual) is safe and therefore should not be withheld from patients. In general, Giovanni Passalacqua concluded that the optimal maintenance regimen (continuous vs precoseasonal) is still an unsolved question and efforts should be made to improve patients' adherence to the AIT schedule.





Food allergy: oral immunotherapy

Oral allergy immunotherapy (OIT) has been mainly applied in the last few years as a treatment for patients with persisting food allergies, including cow's milk, egg, peanuts, tree nuts, wheat, soy, fish and shellfish allergies. Stacie Jones discussed recent randomized controlled trials concerning OIT in patients with peanut, milk and egg allergy, pointing out the essential difference between 'on-therapy' desensitization and 'off-therapy' tolerance. Short-term effects on clinical tolerance; that is, sustained unresponsiveness, are noted after years of OIT in a subset of patients. There are immune changes that support signs of effective immunotherapy, but still no studies fully address long-term tolerance. In this context, Hugh Sampson demonstrated that monitoring IgE and IgG4 epitope-specific antibody levels may be useful in distinguishing patients who are or are not likely to benefit from OIT. It seems that IgE reactivity against sequential epitopes of specific food proteins and, likewise, reactivity against many different epitopes are aggravating factors. Burks *et al.* recently demonstrated that OIT for 22 months applied to 55 children with egg allergy resulted in successful desensitization in 75%, of whom only 28% had sustained unresponsiveness at 24 months [19]. Wayne Shreffler commented on OIT safety in patients with milk and peanut allergy. Regarding milk, a systematic review and meta-analysis by Brożek *et al.* concluded that "a potentially large benefit of OIT in patients with cow's milk allergy may be counterbalanced by frequent and sometimes serious adverse effects" [20], while in peanut allergy the risk of serious adverse reactions prohibits its application outside clinical trial settings. The potential role of pretreatment with anti-IgE has yet to be delineated, with initial trials showing auspicious results [21]. Future directions include optimizing the safety profile of OIT by elucidating the role of cofactors in adverse reaction occurrence and defining the mechanisms of action, the optimal dose and duration of treatment.

Venom immunotherapy

Venom immunotherapy (VIT) is of the utmost importance in cases of anaphylactic

reactions after a hymenopter sting, as well as in cases of coexisting risk factors and/or quality of life impairment. A prerequisite for successful VIT is the correct diagnosis of the culpable insect; mainly honey bee, wasp or polistes. Two groups of patients with a history compatible with insect allergy constitute a diagnostic challenge: those with skin and blood tests negative to both honey bee and wasp (double-negative) and those who test positive against both insects (double-positive). The majority of the lectures and posters in the field dealt with these two groups.

Franziska Rueff and David Golden agreed that patients with concurrent mastocytosis are a challenge since they often present with negative tests despite positive history; this is often overlooked. Baseline serum tryptase measurement helps in the diagnosis of mastocytosis, whereas the basophil activation test can be very informative, since it has a high negative predictive value, a point made by both Gunter Sturm and various posters. Mast cell disorders are a risk factor for anaphylactic reactions, unsuccessful VIT or relapse after VIT and are considered by many as a contraindication to VIT. However, as Hanneke Oude-Elberink and others demonstrated, in such cases, VIT is safe, well tolerated and effective, and should be given for life.

As for respiratory allergy, CRD emerges as an extremely important tool to clear out possible cross-reactivities in the case of double-positive patients. Api m1 is specific for the honey bee, albeit there are geographical differences [22]. By contrast, Ves v1 and Ves v5 are specific for the wasp and, if combined, reach a sensitivity of nearly 95% [23]. All three components should be searched for a suitable VIT. Apart from Api m1, 11 more components have been characterized in honey bee venom. Unfortunately, honey bee extracts used in VIT do not include many of them (e.g., Api m3 and Api m10). Ollert demonstrated that VIT may be unsuccessful for patients sensitized to these components [24]. Spiked extracts with Api m3 and/or Api m10 is a plausible solution in such cases.

As our understanding of mechanisms involved in allergic disease grows, more challenging goals arise. Utilizing novel tools and adopting a more individualized approach may be essential elements in



AIT clinical practice for the future. During the European Academy of Allergology and Clinical Immunology – World Allergy Organization World Allergy and Asthma

Congress in Milan many pieces of these puzzling steps were delineated, although lots of valuable knowledge still eludes us and remains a future challenge.

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