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EDITORIAL

Broad perspectives of allergen specific immunotherapy

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Allergen specific immunotherapy aims to subvert or divert immune responses to allergens to ones that do not cause immunological hypersensitivities. It is performed by the administration of the offending allergen usually in doses low enough not to elicit allergic reactions but high enough to induce protective immune responses or extinguish effector responses. Historically and even today the predominant strategy has been the subcutaneous injection therapy (SCIT) pioneered by Noon and published in 1911 for hay fever induced by pollen allergens. Many people receive benefit from this treatment that can be apparent during a single course of injections and if used for about 5 years can persist without further injections. The protracted injection therapy, the often-incomplete relief, adverse reactions and the expense of medical supervision leave enormous scope for improvements and for food allergens non-injection routes of administration can more effective and safer. Also immunotherapy with allergens other than pollen and for diseases other than rhinitis is not so well developed and might best be accomplished by different procedures. In contrast to pollen-induced rhinitis the symptoms from house dust mite and fungal allergy induced asthma are precipitated by insults such as viral infection on tissue inflamed by chronic allergy instead of acute exposure to allergen so different immunological outcomes might be required. Even within allergies caused by the same allergen recent studies of anti-IgE[1] and anti-cytokine[2] therapies have revealed that the damaging component of the allergic immune response differs between individuals.

As well as the classical SCIT, sublingual immunotherapy (SLIT) for aeroallergens is now well-entrenched as is oral immunotherapy (OIT) for food allergens. It will however be abundantly clear from the papers in this issue that many types of allergen specific immunotherapy are being investigated and that they act by different mechanisms. Knowledge of the different mechanisms of action is important since the lack of definitive biomarkers is a major impediment to ascertaining when immunotherapy has been successful and for selecting subjects that would benefit from different types of immunotherapy. Perhaps relating to the lack of biomarkers many studies still attempt to measure antibody or T-cell responses with allergen extracts that contain highly variable amounts of different allergenic molecules as well as undefined non-allergenic components that impact on the innate immune system. Recombinant allergens are now available for such purposes but even for T-cell studies the purity and structural integrity needs to be established. A new peptide strategy for measuring house dust mite T-cell responses has also been published reporting excellent correlations with IgE titers and Th2 cytokine responses.[3] Selectively interrogating different populations of immune cells is also a challenge but there have been enormous advances in understanding different cell lineages and trafficking and advances in the application of flow cytometry along with gene activation technology such as digital PCR.

The first two papers in this series present snap shots of the use of SCIT and SLIT for two classical sources of allergen namely house dust mites and fungi. Concentrating on the statistical significance of the presence or absence of symptom improvement, Yang and Zhu[4] analyze well controlled trials using house dust mite extracts concluding that they have shown efficacy but because of the absence of standardization, each extract produced needs to be evaluated individually for each targeted patient group. SCIT and to lesser degree SLIT showed efficacy and were considered safe for rhinitis and asthma patients and in a revision of previous recommendations SCIT might be useful for ameliorating atopic dermatitis. Their comments on the transference of studies done mainly in Europe with European reagents from a base in China is very thought-provoking considering the already high burden of HDM allergy in that extremely populous country.

Bozek and Pyrkosz[5] present the daunting task ahead for research to bring immunotherapy for fungal allergens to the state of development for pollen and even house dust mite allergy and, even then, only concentrating on the four most important genera Alternaria, Cladosporium, Aspergillus and Penicilium. A double blind placebo controlled trial and other studies have shown that SCIT with alternaria extract relieved symptoms in alternaria-allergic subjects with rhinitis and asthma although the review points to lack of even medium term data for its longevity. It was concluded that an insufficiency of controlled studies made it impossible to recommend immunotherapy for the other genera and the lack of standardized extracts, especially for Cladosporium were, as also assessed by others, seen as a major impediment. The association of allergy to fungi with severe asthma has been repeated recognized for two decades in several countries and so has the inadequacy of extracts but the subject does not seem to be attracting the attention required to improve its diagnosis and treatment.

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Using tree pollen allergy, especially that to birch and Japanese cedar, Su et al.[6] provide an overview of possible mechanisms of action of immunotherapy and the many ways that allergen administration has been modified most often targeted at removing the protracted therapeutic procedures with their associated expense and poor compliance. Strategies of modifying allergens by conjugation with adjuncts such as microbial pathogen associated molecules to target innate immune receptors are described as well as differentially targeting T and B-cells with modified allergens. These procedures can now to a reasonable extent be accomplished by synthetic peptide chemistry, genetic engineering, DNA vaccines and chemical modification. Many of these procedures have gone to clinical trial including the administration of monophosphoryl lipid A conjugated allergens, Cry j 1-galactomannan conjugates, recombinant birch allergen in SCIT and SLIT, synthetic peptides, overlapping recombinant allergen fragments, a hepatitis B PreS protein-Bet v 1 conjugate, B-cell epitope vaccines and allergens targeted for lysosomal processing by conjugated lysosomal-associated membrane protein-1 (LAMP-1). Oral and epicutaneous and intralymphoid injections have also been trialed. The myriad of ways that immune responses to allergens can be attenuated or re-education has created a rich pipeline for continued advances but the need to conduct clinical trials for valid assessment and the absence of biomarkers has made progress difficult especially when trials have been bedevilled by placebo effects and the variability of real-life exposure to allergen. To add a further problem the lack of published information on critical aspects of some trials make it difficult to assess and improve some strategies. For example it is suspected from an associated study[7] that a failed phase II trial of house dust mite peptide therapy referred to by Su et al. (clinical trial registration NCT02150343) administered a formulation that contained only one peptide representing the immunodominant group 2 allergen of *D. pteronyssinus* and, from the registration of the intended conduct of of the trial, probably compounded this under-representation by trialing patients in countries predominantly infested with *D. farinae*.

The article of Gamazo et al.[8], which explores more than the use of adjuvants encapsulated in the title, continues to summarize methods that could replace the traditional SCIT. It makes the important point that the success of desensitization should not just be a complete lack of response to allergen but include the ability to tolerate increased exposure to allergen. Quantitative provocations are part and parcel of assessments for food and venom immunotherapy but although there are initiatives being undertaken in that direction for aeroallergy, provocation could be more widely used. The ability of new types of immunotherapy to immediately produce clinical improvement to natural exposure is not necessarily the best way to judge progress in developing new strategies and to plan further improvements. The discussion in this paper on the effectiveness of SLIT and the need to establish cogent reasons for using this treatment over the more efficacious SCIT is highly pertinent particularly since although it might have increased the uptake of immunotherapy it has accomplished to opposite for improving patient compliance. The possibility of using new adjuvants is introduced against the background that the mechanisms of action of alum and microcrystaline tyrosine adjuvants that are currently used far exceed any function they might have as repositories for slow release and that their pharmacological effects would almost certainly preclude their ability pass today's safety standards. The development of new adjuvants has been spearheaded by the use of microbial products beginning with the endotoxin component monophosphoryl lipid A for the activation of toll-like receptor (TLR) −4. The summary of patient trials conducted with this adjuvant is a reminder that there were findings of promising clinical efficacy and favorable effects on biomarkers in studies of short course injection regimens conducted 15 years ago[9] but with little follow-up. A strategy targeting another TLR this time TLR9 a ligand for the unmethylated CpG oligodeoxynucleotides has also been examined by clinical trial for ragweed allergy. Injection therapy with a CpG-conjugate with its major allergen Amb a 1 was discontinued because of unfavourable clinical assessments but to add to Gamazo et al.'s review this was also the first large-scale trial of the efficacy of using the single allergen Amb a 1.[10] From the ion-exchange used to prepare Amb a 1 it was unlikely to adequately represent all of its isoforms which, with their sequence disparity of 25%, need to be regarded as separate allergen components. Further, despite some uncritical promulgations of the importance of Amb a 1 its contribution to the anti-ragweed IgE titers of different patients varies in an even continuum of proportions ranging from 25%–85%. Moving from microbial products and their derivations the exposition of nanoparticles and their potential to deliver allergens and be made to interact with pattern recognition receptors on antigen presenting cells show the almost unlimited possibilities now in the hands of researchers, emphasizing the need to consider how they can all be evaluated.

Bacterial ghosts offer a little-considered option for immunotherapy warranting their separate consideration in this issue.[11] They can be produced from a wide range of bacteria infected with plasmids that direct the production of a cell-wall-puncturing bacteriophage protein. The bacterial shells offer a defined and highly manipulatable delivery system that can be exploited by the many laboratories competent in basic recombinant techniques. Recombinant proteins can be expressed on surface of the ghosts that retain their native cell-wall structure and can be adjuvanted by the surface expression of proteins that target antigen presenting cells and innate immune receptors. If administered in similar doses to those now used for bacterial vaccines[12] the expression of up to 100 000 copies of surface allergen per cell ghost would however limit the therapies to ones that depend on nanogram dose strategies. Similarly while proteins expressed in the periplasmic are also retained in ghosts the levels of expression, of about 5% of the bacterial protein, achieved by this method imposes similar limitations.[13] The advantages proposed for the ghosts however should be considered because they include sequestration from IgE bound on mast cells and basophils and degradation as well the ability to target antigen presenting cells.

Chen and Land[14] introduce platform concepts for food allergy immunotherapy. The choice of the delivery being considered today is between the oral, sublingual and epicutaneous routes with subcutaneous injections generally eschewed because of safety concerns. The clear winner is oral immunotherapy, which although it produces adverse effects they are, as

found for SCIT for venom and aeroallergens, associated with greater efficacy. Oral tolerance also not only provided one of the first demonstrations of immunological unresponsiveness but that feeding induced a mixture of effector and different types of regulatory cells.[15] Combinations of sublingual, oral and epicutaneous are nevertheless under investigation with a view to ameliorating adverse effects and to help patients achieve higher maintenance doses more rapidly. A further combination considered is that with probiotics as studied with peanut allergy. As presented a trial has shown short-term efficacy but did not make a comparison with OIT alone. A paper published after the submission of this article[16] reports a follow up undertaken 4 years after the cessation of the trial. It found with samples of 24 subjects that the group that received the probiotic were significantly more likely than the placebo group to have continued eating peanut and had smaller wheals in skin prick tests and higher peanut sIgG4: sIgE antibody ratios. There was however a reluctance of the participants, including those in the probiotic treatment group, to undergo controlled challenge so this needs further investigation. Despite the statistical success of the oral immunotherapy trials there is dearth of follow up and the role of continued ingestion or "natural maintenance" is still uncertain. The limited observations[17] suggest only limited success without continued maintenance. Milk and egg allergy also have the advantage that their allergens can be modified by cooking and by hydrolysis for example by renin, producing peptides with reduced IgE activity. Little has been followed up with peptides but cooked egg and milk allergens are being investigated as modified allergens to aid the induction and maintenance of immunotherapy. The anti-IgE monoclonal antibody omalizumab being explored as an adjunct to oral immunotherapy has shown promising potential with peanut and milk and egg to help patients reach higher maintenance doses faster and with fewer adverse, although the end outcomes have been similar. While this appears to be an advantage the costs of the drug needs to be considered as well as longer-term outcomes.

Taniuchi et al.[18] provide an account of cow's milk immunotherapy focusing on oral tolerance. It proposes from clinical trials that for most patients one of two strategies could be used, being either a three-step desensitization procedure consisting of rush, build up and maintenance or for mildly allergic patients a two-step rush and maintenance procedure. Alternative protocols might however be needed for complete desensitization and for patients with very severe allergy. Combinations with SLIT and epicutaneous therapy, which might induce regulatory T cells and immunosuppressive cytokines were considered. From a global perspective it was noted that despite the regular use of oral immunotherapy for young children in several countries there are no standard protocols and studies with long-term follow-up to observe sustained unresponsiveness are rare. These concerns were echoed by Graham et al.[19] for egg allergy noting that oral immunotherapy has produced clinical tolerance that can persist for as long as it is regularly ingested. Evaluations of sustainability have shown, depending on the study, that lapses of 4 weeks to 6 months in exposure break the clinical tolerance. Data on changes in biomarkers during the oral immunotherapy show variable effects on IgE antibody titers but with more consistent reductions in skin test responses and increased IgG4 antibody. Limited data for T-cell responses show skewing away from a Th2 phenotype. A measure for sustained responsiveness to egg oral immunotherapy that could have considerable clinical utility has been found in increased IgG4 and IgA antibody titers to ovomucoid and should be subject to further investigation.

In contrast to cow's milk, egg and peanut allergy, immunotherapy for wheat allergy and has received little consideration. Pacharn and Vichyanond[20] document that it is a far more common cause of anaphylaxis than recognized, especially among young children and the high prevalence in an Asian country might be counter to common expectations. In the USA and Thailand it has been reported to mostly resolve by 6 years of age but for the USA can persist into the teens for 35% of cases. Wheat avoidance is not easy to follow, because it is a common constituent of many processed foods. Only a few clinical trials of oral immunotherapy have been conducted. The reagent of choice appears to be pasta although porridge and flour in tomato sauce has been used. The trials could achieve a clinical tolerance for most subjects after a 6-month treatment but this was lost on cessation of treatment. Even with maintenance therapy about a 60% success was found over 2 years. The report here thus provides a timely account of an allergy for which further clinical and immunological studies of immunotherapy are required including the conduct of randomized, placebo-controlled trails. From an immediate perspective Pacharn and Vichyanond emphasize the need to determine more precise estimates of the duration of the current treatments required to produce clinical tolerance and the maintenance dosing required to sustain it.

Stinging insect venom allergy has a special place in the history of subcutaneous injection immunotherapy with a long-standing recognition of being a successful treatment for a life-threatening condition. Since bee stings can kill allergic subjects within a few minutes the treatment has not only been medically effective but has relieved the anguish of many sufferers and their families. It however should not go unnoticed that, by the assessment of Schiener et al.[21], even after 5 years of therapy all patients continue to have a 10% chance of having a systemic reaction to a future sting, even if venom skin tests become negative. Sting challenges in older studies have shown failure rates of up to 22% of patients. As with other allergies the lack of biomarkers to show successful treatment is a major issue. A milestone discovery however has been to identify that patients with a predominant sensitization to the allergen component Api m 10 have a high risk of treatment failure, with this allergen only being present in venom and venom-therapy products in small amounts. It is also noted that a drawback of most studies is the lack of sting challenge data during and after therapy to correlate with any of the biomarkers. Schiener et al. describe experiences with omalizumab that could have ready application in reducing the painful large local reactions at injection sites found for 10–15% of subjects. Options for the use of adjuvants, modified allergens and novel delivery systems, similar to food and aeroallergens are ventilated in the article describing the paucity of research into their application.

Bee venom immunotherapy is also famous because much of its success was attributed to injecting venom instead bee extract. While this makes a great deal of sense the older

trials also paid less attention to the clinical assessment of the subjects. The understanding of bee allergy was further hastened when it was realized that much of the serum IgE binding to venom components was via cross reactive carbohydrate residues that did not mediate allergic reactions and are induced by glycoproteins from a variety of sources including pollen. Recombinant allergens can now be used to ascertain the correct allergic responses. The poor responsiveness of high responders to Api m10 to immunotherapy shows the importance of profiling the allergen components that induce the allergy and since this is in its infancy more useful revelations might be expected. Blank et al.[22] summarize the knowledge of the important bee venom allergens Api m 1, 2, 3, 5 and 10 with only Api m 1 being present in high quantity. The balancing act required to induce unresponsiveness to Api m 2, 3,5 and 10 in the face of unwanted allergic reactions to Ap1 m 1 can readily be appreciated and for Api m 10 the task would be more difficult because it is often not found in venom preparations. Such imbalance makes a mockery of the current so-called biological standardizations where skin test reactivity to an undefined mixture of allergens is used to determine the dose at which it is used without reference to the allergen components. Although the use of specific antibodies to monitor extracts described here can be used for formulations the use of recombinant allergens might be more realistic. The elucidation of the spectrum of bee venom allergens and the potential of this knowledge has a parallel in cat allergy once thought to be dominated by one allergen but now shown to be more complex with patients having qualitatively different allergen-recognition profiles.[23]

The studies of immunotherapy for house dust mite, fungus, egg, milk, wheat and venom give a broad perspective of the challenges required to improve immunotherapy for each allergen source. More knowledge of the allergic responses that underpin both the induction and expression the allergies is sorely needed but it is only slowly being appreciated that, like immunological studies in mice, it is necessary to use defined reagents in defined doses for authenticatable observations and to make them in the absence of substances in allergen extracts that make varying and unknown interactions with elements of the innate immune system. Nevertheless the data to date shows that improvements in allergen immunotherapy can all be pursued in the knowledge that the principle that it can produce clinical benefit has been established. Critical for the concept of allergen-induced changes it has in some cases been accomplished with pure allergen.[24] The enormous scope for improvement in its efficacy, the method of induction and the establishment of biomarkers has just as clearly been shown. The astonishing results in recent cancer research has demonstrated not only the enormous power of immune checkpoints in restraining powerful anti-cancer immune responses in humans but that they are evoked in immune responses to many different types of tumours that would have different antigens. In a reverse strategy the activation of these and other checkpoints combined with antigen administration is already being considered for autoimmunity and holds promise for similar advancements in allergic disease.

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