Safety and efficacy of polymerized extracts for immunotherapy

IgE-mediated allergy is a global problem affecting more than 20% of the population in industrialized countries. In contrast to the merely symptomatic treatments, specific immunotherapy (SIT) is the only prophylactic desensitizing therapy for allergy. On the other hand, the potential for local and systemic reactions has forced to improve the traditional use of conventional allergen extracts. First of all, standardized extracts have improved the reliability of SIT. Another safe approach was the change of the extracts' administration route (i.e. sublingual). Finally, a different approach involves the chemical modification of allergens in order to reduce their allergenicity (ability to elicit IgE mediated reactions) by destroying conformational B cell allergen epitopes while maintaining their immunogenicity (induction of IgG antibodies and modulation of the immunological response) by preserving linear T cell allergen epitopes. Glutaraldehyde (GA) is a bifunctional aldehyde that acts as a protein cross-linker producing high-molecular-weight allergen polymers. The allergenicity of polymerized extracts diminishes because larger molecules diffuse more slowly and contain more concealed antigenic determinants, which are preferentially processed by phagocytic cells (monocytes, macrophages and dendritic cells) and could still transmit complete immunological information to antibody-producing cells. Finally, polymerized allergens have a lower molar concentration than the same weight of native proteins and thus a decreased opportunity to bridge IgE on the surface of the mast cells and cause mediator release. Moreover, modified allergens lacking B cell epitopes prevent IgE binding and effective cell cross-linking and do not employ IgE mediated antigen presentation. For example, GA-polymerized ovalbumin selectively induced a Th1-like cytokine synthesis pattern in mice. As a result of polymerization, the proteolytic rate of polymerized allergens diminished compared with that of the monomer, and therefore provided a slowly degraded depot antigen. Thus, since polymerized allergen extracts are safer, they can be administered with fewer injections over a shorter time period with a lower risk of systemic reactions, and decreasing the cost and inconvenience to the patient in terms of physician visits, and therefore improving patient compliance. The efficacy of SIT with chemically modified allergens has been verified by numerous clinical trials. Some published papers covering our experience can be remarked. The model that we have studied in a deepest detail is the polymerized extract from the pollen of *Parietaria judaica.* The production and characterization of this polymer was reported in 2004 (I. Ibarrola *et al*. Biological characterization of glutaraldehyde-modified *Parietaria judaica* pollen extracts. *Clin Exp Allergy* 34:303–309) to show the array of techniques use to ascertain the polymerization processes (free amino group determination, high performance liquid chromatography, SDS-PAGE) and to verify the reduction of allergenicity (cutaneous tests, histamine release, IgE binding methods) and the maintenance of immunogenicity (PBMC proliferation assay, IgG induction). Nowadays further methods can contribute to the extensive control of the polymerized extracts, such as mass spectrometry, ELISA using discriminating antibodies, light scattering, SEC, etc.

Tolerance and immunological changes of chemically modified allergen vaccine of *Parietaria judaica* in accelerated schedules

A clinical trial with *P. judaica* polymers was conducted to demonstrate their tolerability, efficacy and the key fact of induction against the major allergens Par j 1 and Par j 2, thus revealing that although these allergens cannot be detected by conventional ELISAs they are present in the polymerized extracts (J.A. Asturias *et al*. Tolerance and immunological changes of chemically modified allergen vaccine of *Parietaria judaica* in accelerated schedules. Clin Exp Immunol, 147:491-496). The physicochemical modification of allergen vaccines provides a chance for administering higher doses in a shorter period of time. In this study we sought to assess the safety and immunological changes of using a biologically standardized and modified *P. judaica* pollen extract in accelerated schedules. Two accelerated schedules were tested in *45 P. judaica*-allergic patients: 20 patients reached the maximum dose after two visits using two different concentrations and 25 patients reached the maximum dose after only one visit with two injections of the maximum concentration vial. The tolerance was assessed by recording all side effects related with immunotherapy. Specific antibody levels against native extract and rPar j 2 allergen were evaluated at the beginning and the end of the study. Allergenic potency determined by enzyme allergosorbent test (EAST) inhibition and skin prick test showed that modified *P. judaica* pollen had a 99·9% less allergenicity than native extract. After 650 doses administered, two clinically irrelevant local reactions (diameter < 0·5 cm) and no systemic reactions were registered. Significant increases in allergen-specific IgG4 and IgG against *P. judaica* extract and rPar j 2 and significant decrease of specific IgE against Par j 2 were observed. The modified extract of *P. judaica* is safe to treat sensitive patients, even at accelerated regimens, and induces significant immunological changes.

Tolerance of chemically modified allergenic extracts administered under an ultra-rush schedule

The observations found with *P. judaica* were further extended to a variety of extracts from different sources (Tolerance of chemically modified allergenic extracts administered under an ultrarush schedule. B. Madariaga *et al*. *Allergy* 63 (Suppl. 88) 158-611). In this work, is assessed the tolerance of different polymerized allergen extracts under an ultra-rush schedule.

Biologically standardized polymerized extracts of *Dermatophagoides* mites and pollens from grasses, *Olea europaea*, *Parietaria judaica*, *Cupressus arizonica* and *Platanus acerifolia*, were used to produce the vaccines. One hundred and forty one subjects received immunotherapy with those preparations. Ultra rush

schedule consisted in the administration of 0.2mL and 0.3 mL, one in each arm, within 30 min intervals, in a unique day. The first maintenance dose was administered 1 month later, and so on at monthly intervals. Adverse events were registered and graded according to EAACI. Twelve patients out of one hundred and forty one were excluded due to the use of vaccines not allowed in the study (mixtures). There was no systemic reaction in any patient treated with polymerized immunotherapy. Only six subjects (4.7% of patients) showed local reactions with a wheal diameter < 5cm in the induction phase, that is, in the first day. Over the whole period (6 months), seven patients experienced some kind of local reaction (wheal and/or itching) all of them minor than 5 cm, that is 1.43% of the doses. The responsible extracts were *Parietaria judaica* in one case, grasses in two cases and mites in four cases. There were no with-drawals due to local reactions. The study concluded that SCIT vaccines with polymerized extracts showed an acceptable safety profile administered by an ultra-rush schedule.