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Measurement of immunoglobulin E interaction with allergen extracts by surface plasmon resonance biosensor analysis

The safety of chemically modified allergen extracts (allergoids) is well documented. The rationale behind allergoids is to reduce allergenicity[1] by blocking or modifying the conformational epitopes or hiding the sequential epitopes of allergens. The resulting product consists of a chain of cross-linked allergens, with high molecular weight and a significant reduction of free amino groups. These products have been used in DBPCCP, showing clinical efficacy, high safety profile,[2] and immunological changes.[3] The immunoglobulin E (IgE)-binding capacity of allergenic extracts and, therefore, the measurement of vaccine safety are currently estimated by indirect methods, largely enzyme-linked immunosorbent assay inhibition experiments, which consist of establishing the 50% inhibition point of the extract relative to an in-house reference preparation.[4] In the case of allergoids, the reduction in IgE-binding capacity is defined as the percentage reduction of the new synthesized molecule relative to the previously polymerized extract. However, the percentage reduction only provides an idea of IgE-binding capacity relative to native extracts.

Surface plasmon resonance (SPR) biosensors enable the measurement of molecular interactions in any sample (proteins, peptides, carbohydrates, lipids, drugs, and so forth), characterizing binding reactions in real time with no labeling requirements.[5] The technique is based on immobilizing the molecule (the ligand) on a sensor chip and monitoring its interaction with a second component in solution (the analyte). The SPR biosensor used measures the angle shift of the SPR dip. The sensorgram is the plot of the minimum of the SPR angle shift as a function of time. This is a consequence of the change in the solvent's refractive index near the surface that occurs during complex formation or dissociation.[6] In recent years, SPR technology has emerged as a key technology for the measurement of antigen–antibody interaction,[7] but it has not been extensively applied to the field of allergy. SPR has been used for the detection of free IgE,[8] for the determination of the affinity of recombinant antibodies for recombinant allergens,[9,10] or for food allergen detection.[11] This method enables quantification of the reduction in activity by measuring the affinity of the allergen extracts to IgE and evaluating the kinetics of the antigen–antibody reaction. The objective of this preliminary study was therefore to use SPR to evaluate the reduction of allergenicity of a depigmented polymerized (Dpg-Pol) allergoid extract of *Dermatophagoides pteronyssinus,* comparing the stability of the IgE-allergen with respect to the corresponding native extract.

The SPR measurements were performed on a Biacore T100. A preconditioned C1 chip (GE Healthcare, Piscataway, New Jersey) was used. Monoclonal anti-human IgE (Ingenasa, Madrid, Spain) was diluted to 20 μg/mL in 10 mM sodium acetate, pH 5, and immobilized onto flow cells 2 and 4 as described in the Sensor Surface Handbook (Biacore; GE Healthcare), obtaining a response of 1,590 (Fig. 1A) and 939 response units (RU), respectively. The reference flow cell was treated in the same way but without immunoglobulin. Flow cell 3 was kept intact to see possible nonspecific binding to the surface (not observed). The second step consisted of capturing IgE from a serum pool manufactured by mixing the same volume of 10 individual serum samples from individuals allergic to *D. pteronyssinus* (Plasmalab, Everett, Washington). The pool of sera (diluted 1:2 with HBSEP containing 10 mM N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid), 150 mM NaCl, 3 mM ethylenediaminetetra-acetic acid, 0.05% Tween20) was injected for 350 seconds at a flow rate of 2 μL/min, to capture IgE (Fig 1B). Finally, native and Dpg-Pol *D. pteronyssinus* (Laboratorios LETI, Madrid, Spain) were injected at 30 μL/minute for 120 seconds at 2 different concentrations (0.2 and 0.4 mg/mL in HBSEP). The dissociation phase (Fig 1C) was recorded for 1,800 seconds to measure the half-life of the complex ($t½ = \ln 2/k_d$). Regeneration between the different cycles was obtained with 20 mM Glycine at pH 2 during 120 seconds at 30 μL/min. This methodology informs us of the relaxation time of a receptor ligand multiplied by 0.693 ($-\ln[0.5]$) and thereby relates this half-life (half-life = 0.693/kd).[7]

Dpg-Pol showed a significantly higher Kd and lower t½ than native extract. In the first assay (RU immobilized anti-IgE: 1590), kd for native was 3.531E-05/second and t½ 19,628 seconds, whereas for Dpg-Pol it was 1.25E-04/second and 5522.8 seconds, respectively. When response of the immobilized anti-IgE was 939 RU, the results were Kd 2.54E-05/second and t½ 27,265 seconds for native extract and 1.26E-04/second and 5489.3 seconds, respectively, for Dpg-Pol extract. Thus there were no differences between the 2 assays, showing that different densities of immobilized anti-IgE do not influence the results.

In this preliminary study, we propose a new methodology for evaluating IgE–allergen extract interaction that is an essential prerequisite for evaluating the clinical safety of allergenic vaccines. SPR is currently being used for the characterization of IgE–antigen (allergen) interaction, using monoclonal antibodies or recombinant or purified allergens.[9,10] However, our objective was to evaluate the residence time for a complex mixture of allergenic and nonallergenic components (allergen extract) and polyclonal IgE (from the serum of patients containing other antibodies, such us IgGs), comparing 2 types of extracts to determine the stability of the complex against the IgE.

The complexity of the challenge led us to a 3-step design in which anti-IgE is immobilized and then binds to IgE, which binds to IgE-epitopes from allergen extracts. We are aware that by including an additional step the results could be more difficult to interpret,

Disclosures: Authors have nothing to disclose.
Funding Sources: This study was funded by Laboratorios Leti, Spain.

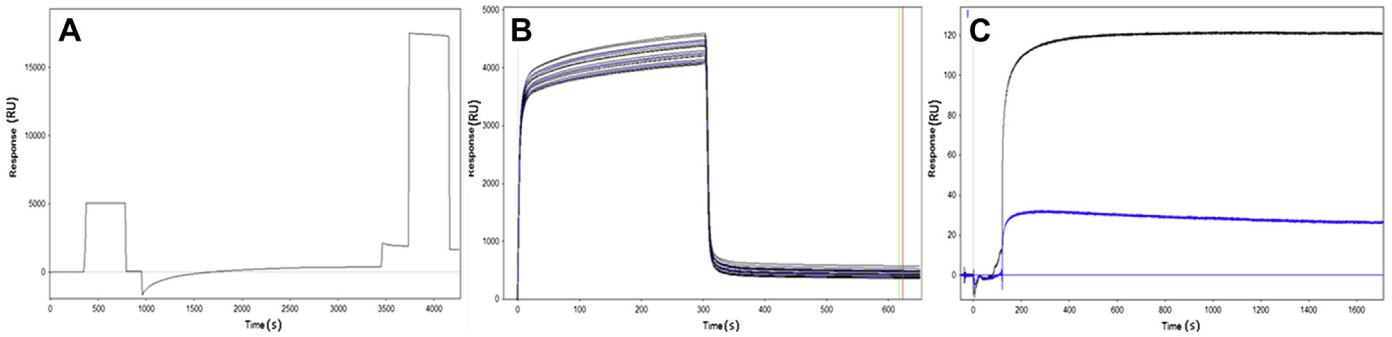

Figure 1. (A) Immobilization of anti IgE antibody at 1,590 RU. (B) Capture of IgE at different dilution of pool of sera by immobilized anti IgE. (C) Sensorgram of the interaction between IgE and native (LN; *blue line*) and depigmented-polymerized (LP; *black line*) extracts of *D. pteronyssinus*.

because we are simultaneously measuring the binding strength of the allergen to IgE and that of the IgE to the anti-IgE. However, we can assume that the off rate of IgE for anti-IgE is the same, and that the differences between both extracts are attributable exclusively to the IgE–allergen extract interaction.

This is the first time that IgE stability for allergenic extracts has been measured in real time, providing an idea of the reactions' kinetics. Moreover, results obtained through SPR have been confirmed with those obtained using the current immunoassay (enzyme-linked immunosorbent assay inhibition), showing that Dpg-Pol extracts have a reduced IgE-binding capacity. More in-depth studies are needed to establish the relationship between clinical safety and the reduction of IgE affinity.

In conclusion, the SPR assay described in this study is an adaptation of the classical assay based on a system that quantifies and measures the kinetic reaction between antigen and antibody, which is similar to that used for the analysis of the interaction between monoclonal antibodies and their respective antigens. It is important to highlight the role of the dissociation rates, which are independent of the analyte concentration. The main advantage of this method involves measuring the real-time IgE–allergen interaction qualitatively and quantitatively. However, these are preliminary results, and further exhaustive studies are needed for it to be used as a routine method for allergenic vaccine characterization. We believe that the method can be used not only for the determination of allergenic vaccines but also for the design of new immunotherapy systems that guarantee patient safety.

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Association of subclinical hypothalamic-pituitary-adrenal axis suppression with bone loss in patients with asthma taking inhaled corticosteroids

Studies on the systemic effects of inhaled corticosteroids (ICS) have primarily focused on suppression of the hypothalamic-pituitary-adrenal (HPA) axis, yielding conflicting results,[1,2] perhaps because of differences in the types and doses of ICS, concomitant use of oral corticosteroids, or the methods used to evaluate the HPA axis. The adrenocorticotropin (ACTH) test is a common screening test for evaluating it. Compared with the conventional ACTH test using a supraphysiological dose (250 μg), the low-dose ACTH test (1 or 0.5 μg) is more sensitive for the assessment of the axis.[3,4] By the low-dose ACTH test, some patients with asthma taking ICS have been identified as having subclinical HPA axis suppression.[1,2] However, whether this suppression is associated with pathological conditions in such patients remains unknown. We evaluated the relationship between subclinical HPA axis suppression

Disclosures: Authors have nothing to disclose.