Characterization and recombinant allergen production, immunotoxin production and mRNA vaccine as diagnostic and therapeutic tools in allergy

Brief summary

Allergy is a major health problem, already affecting more than 30% of the population in developed countries. Initially, our research was based on the study of the physicochemical and immunological characteristics of the molecules that cause allergies, the allergens, and the approach to their recombinant production to obtain quantities of these molecules with sufficiently good quality to be marketed as diagnostic and therapeutic tools. In recent years, allergy has been redefined not only as an alteration in the patient's immune response but also as a dysfunction of the epithelial barrier that acts as an interface between our internal and external environments and that facilitates, or not, the access of allergenic molecules found in the organism's environment. In the case of asthmatic patients, an alteration in the respiratory epithelial barrier has been observed and we have shown that the same occurs with the intestinal epithelium in food allergies. The study of how the physiological state of the epithelium influences the immune response to aeroallergens or food allergens, and which are the factors that act synergistically with allergens, as well as the involvement in the interaction of pulmonary surfactant -the lipid-protein mixture that coats the alveoli of the lungs- are some of our lines of research that have required the development of new cell culture and microscopy techniques. We use several techniques, including large-scale proteomics and genomics, to obtain a differential profile of proteins, mRNA and miRNA that will allow us to deepen our knowledge of the allergic response and thus be able to design new therapeutic strategies for its treatment.

Our possible collaboration role would focus on the adaptation of these systems to the client's needs; that is, designing the protocols for the isolation of the protein according to the quality or quantity requirements that we are informed of and, if required, the generation of specific antibodies against said protein. Also, the production, mutagenesis and obtaining of modified proteins, of analogous derivatives to the natural protein that respond to the client's specific needs, their recombinant production and the possibility of advice for the incorporation of the methodology to the contracting company.

How it works ?

We purify proteins from different biological materials - pollen, plant foods and mites -, the proteins responsible for the sensitization of allergic patients. In the laboratory we have more than 30 years of experience in the purification and characterization of proteins, using conventional chromatographic systems and HPLC or FPLC. By means of two-dimensional electrophoresis we can determine the molecular mass, the isoelectric point and the degree of polymorphism of a particular allergen. We use immunological (ELISA and Western blot) and spectroscopic (UV/Vis absorption, circular dichroism and fluorescence emission) techniques. Using biophysical techniques we determine their biochemical activity, such as ligand binding or the presence of oligosaccharides in their structure.

We produce recombinant allergens from their corresponding cDNA, previously amplified by PCR, and inserted into a gene vector that serves as an expression vehicle. Once the host cells (generally bacteria - *Escherichia coli* - or yeast - *K. phaffii* -, insect cells - via infection with Baculovirus - or plants -*Nicotiana benthamiana*-) have been transformed with the vector, the culture is grown until it reaches adequate production levels of the recombinant protein.

The protein thus produced must be purified to homogeneity, making use of its chemical-physical properties, molecular mass, solubility, charge, among others. For the recombinant protein, to effectively replace the protein obtained from the natural biological source, it is essential to verify that its molecular properties are equivalent. For this reason, the structural and functional characterization of the recombinant protein is a critical step in assessing the expression system used.

We design and produce polyclonal antibodies to perform the validation of allergens, both natural and recombinant.

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We design and produce recombinant immunotoxins based on ribotoxins, aimed at immunomodulating the allergic response by destroying those cell types against which they are directed. We use allergens, cytokines or antibodies against cell receptors as marker domains.

What problem does it solve?

a) It enhances molecular diagnostics with optimized allergens and extracts. Until relatively recently, diagnostic tests were performed almost exclusively with biological extracts that constituted heterogeneous mixtures of proteins and other non-protein compounds. In recent years, molecular diagnosis carried out with purified allergens, either natural or recombinant, has largely solved the initial problems, but its cost and the complexity of its development prevent it from being a diagnostic test that is available to the entire population. We are a world leading group in molecular allergology having identified more than 50 allergens responsible for respiratory and food allergies, which have allowed us to evaluate the agent causing sensitization and associate it with the clinical symptoms of patients. Therefore, we have extensive experience in detection (including hidden allergens), characterization and purification of proteins of interest for clinical use. From the purified proteins we obtain antibodies of high quality and specificity for use in the validation and quantification of allergens in biosanitary products such as vaccines.

b) Production and validation of recombinant allergens. Another important problem to be solved is obtaining quality allergens in such quantities that they can be introduced into the pharmaceutical market and used at the clinical level. The use of bacteria (Escherichia coli), yeasts (*Komagataella phaffii*, formerly Pichia pastoris) or tobacco plants (*Nicotiana benthamiana*) makes it possible to produce and purify high quality allergens in abundant quantities. In addition, the aim is to improve the production and quality of allergens in safer and more sustainable expression systems for molecular diagnostics. Plant systems, such as the tobacco plant, stand out for their low cost, high scalability, lower risk of infection and purification efficiency, in addition to allowing key post-translational modifications for the biological activity of these proteins.

c) Reduce the number of allergens needed for molecular diagnostics by designing consensus allergens. Produce consensus recombinant allergens obtained by rational design from the aligned sequences of proteins of the same family from different species, with diagnostic and therapeutic applications. These allergens (we could call them Frankenstein allergens) can agglutinate all the allergenic epitopes of the proteins of the same allergen family.

d) Recognition by immunochemical techniques by obtaining polyclonal antibodies against allergens. Our group aims to obtain polyclonal antibodies in rabbits of all the allergens in our collection to quantify and detect them. To date, more than 300 antibodies have been obtained, and agreements have been established with different companies.

e) The development of cellular models of bronchial and intestinal epithelia. We have cellular model systems that mimic human bronchial and intestinal epithelia in different stages of differentiation. We have been able to establish models of chronic epithelial damage that simulate the damage induced by environmental agents and allergens. The group has developed assays for epithelial capture/permeability, cell toxicity, protein expression and subcellular localization, and multiomic analysis (transcriptomics and metabolomics, etc.) with the collaboration of the UCM research assistance centers.

f) Improve treatments through the development of new therapeutic strategies: immunotoxins and mRNA vaccines. Our group has extensive experience in the design, production and characterization of recombinant immunotoxins based on ribotoxins, initially directed against cancer and currently to suppress or immunomodulate the allergic response.

What future products will result in?

We will have optimized extracts of the different allergenic sources, allergens purified from the natural source, recombinant allergens in the expression system that is most appropriate for each protein, chimeras of allergenic

families to have consensus allergens that can bind the epitopes of all the molecules of the same family, immunotoxins containing a specificity marker against specific allergy molecules and cells and an effector domain that destroys these specific cells, and finally the production of polyclonal antibodies.

Competitive advantages compared to another research

The purification of allergens and their recombinant production provides a system for obtaining unlimited highly homogeneous proteins whose presence in nature is scarce, unstable or, in any case, whose isolation involves difficulties. In addition, fragments or, by directed mutagenesis, derivatives of these molecules with modified stability properties or biological activity can be prepared. Our research has resulted in a more potent and effective diagnostic for allergic patients.

Where was it developed?

These technologies have been developed in the Department of Biochemistry and Molecular Biology of the Faculty of Chemical Sciences of the Complutense University of Madrid. More than 20 proteins have been produced in *E. coli*, more than 15 in *P. pastoris* and Baculovirus and, recently, also in *Nicotiana benthamiana*. These allergens have been purified, and analysis of their structural and functional properties has shown that they are equivalent to the corresponding forms produced in nature.

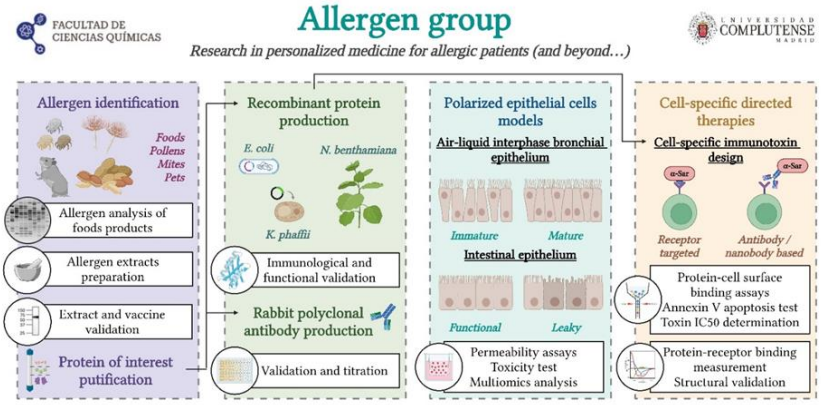

Figure 1. *Lines of research of the Allergen group*

And besides…

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