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Multiple IgE recognition on the major allergen of the *Parietaria* pollen Par j 2

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ABSTRACT

The interaction between IgE antibodies and allergens is a key event in triggering an allergic reaction. The characterization of this region provides information of paramount importance for diagnosis and therapy. Par j 2 Lipid Transfer Protein is one of the most important allergens in southern Europe and a well-established marker of sensitization in *Parietaria* pollen allergy. The main aim of this study was to map the IgE binding regions of this allergen and to study the pattern of reactivity of individual *Parietaria*-allergic patients. By means of gene fragmentation, six overlapping peptides were expressed in *Escherichia coli*, and their IgE binding activity was evaluated by immunoblotting in a cohort of 79 *Parietaria*-allergic patients. Our results showed that Pj-allergic patients display a heterogeneous pattern of IgE binding to the different recombinant fragments, and that patients reacted simultaneously against several protein domains spread all the over the molecule, even in fragments which do not contain structural features resembling the native allergen. Our results reveal the presence of a large number of linear and conformational epitopes on the Par j 2 sequence, which probably explains the high allergenic activity of this allergen.

1. Introduction

An allergic reaction is initiated by a series of cellular events which are primarily mediated by the interaction between IgE antibodies and allergens. The major event is the cross-linking of IgE on mast cells and basophils, leading to the release of the inflammatory mediators responsible for the immediate reaction, but other immunological mechanisms involving IgE antibodies have also been described (Acharya et al., 2010; van der Heijden et al., 1993; Ying et al., 2001). However, despite the pivotal role established for IgE in allergic reactions, little is known about the interaction between allergens and antibodies, mainly due to the low concentration of this class of immunoglobulin in the sera of patients and to the fact that IgE-producing B cells have been poorly characterized [reviewed by (Gadermaier et al., 2014)]. Most of the data related to this aspect are derived from *in vitro* assays using mouse monoclonal antibodies and/or recombinant chimeric human IgE derived from mouse antibodies (Tai et al., 2013; Tiwari et al., 2012). In this respect, it has been demonstrated that human allergen-specific IgE may interact in a different way from mouse antibodies (Aalberse and Crameri, 2011); therefore, these strategies may not be able to fully elucidate the overall spectrum of antibody recognition. In addition, starting from the observation that the degree of effector cell degranulation is determined by IgE concentration and the number of recognized epitopes (Gieras et al., 2007), this information can be relevant for any strategy targeting IgE/allergen recognition.

In this context, little is known about the Lipid Transfer Protein (LTP) family of allergens. LTPs are one of the more relevant classes of allergenic proteins in pollen and plant-derived food (Campana et al., 2011). These allergens are characterized by heat resistance and stability at acidic pH as a consequence of their compact, highly conserved three-dimensional structure, characterized by the presence of a conserved pattern of cysteine residues that forms four disulphide bonds compacting four alpha-helices. This overall fold has been detected in all the available structures of allergens belonging to this family (Pfam database PF00234). These features make this family of allergens of particular clinical interest since recent studies have shown that, in food-allergic patients, the majority of anaphylactic episodes occur in patients sensitized to LTPs (Asero et al., 2009).

Parietaria judaica (Pj) pollen is one of the major outdoor allergenic sources in the Mediterranean area, with two major allergens

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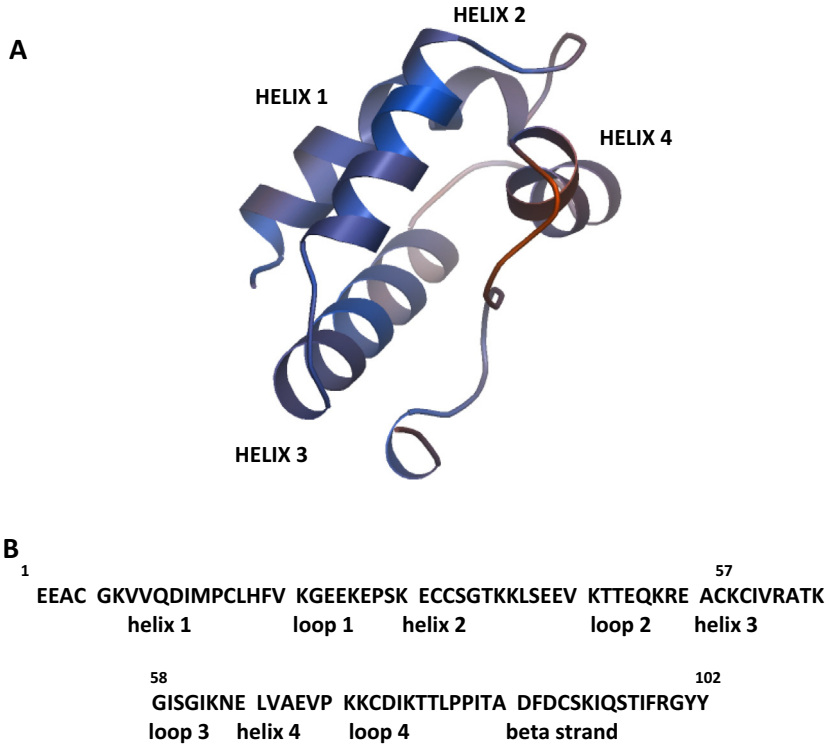

Fig. 1. 3D model by homology. Panel A: Ribbon representation of the Par j 2 allergen determined by using the Swiss-Model Protein Modelling Server using the PDB entry 1FK1A as a template. Panel B: Mapping of the predicted α-helices and loops on the Par j 2 sequence.

which both belong to the LTP family (Par j 1 and Par j 2) (Colombo et al., 2003). In particular, the Par j 2 allergen has been recognized as the specie-specific allergen marker of Pj sensitization (Stumvoll et al., 2003) whose 3D model and disulphide bond assignment has been determined (Amoresano et al., 2003; Colombo et al., 1998). Some preliminary studies have been performed attempting to define B cell epitopes on both the Par j 1 and Par j 2 allergens (Asturias et al., 2003; Colombo et al., 1998; Costa et al., 2000).

In this study, we analyzed the IgE binding activity of six overlapping regions of the Par j 2 allergen by using a set of 79 sera from *Parietaria*-allergic patients. This study allowed us to define a large set of IgE binding epitopes on a relatively small allergenic protein such as the Par j 2, which can explain the high allergenic potency of this protein and open the way to a rational approach for the design of hypoallergenic derivatives for the members of this family.

2. Materials and methods

2.1. Study population

Between November 2005 and May 2006, 2150 children (10–17 years old) living in Palermo, in the Mediterranean area of southern Italy, completed a questionnaire based on SIDRIA and ISAAC surveys and underwent skin prick tests (SPT) at school (Cibella et al., 2011). Skin prick tests were performed according to EAACI recommendations, with a standard panel including *Dermatophagoides* mix, grass mix, *P. judaica*, olive, dog and cat dander, alternaria, and *Blattella germanica*, plus a positive (histamine 1%) and a negative (saline) control (Stallergènes Italia S.r.l., Milan, Italy). The reading was performed after 15 min: reactions were considered positive if the mean wheal diameter (computed as the maximum diameter plus its orthogonal divided by 2) was 3 mm or greater, after having subtracted the wheal diameter of the reaction to the negative control. Allergic sensitization was defined as the presence of at least one positive skin prick test. For the purpose of the present study, all the 311 children showing allergic sensitization for *P. judaica* were recalled for further investigation. The study was approved by the Institutional Ethics Committee. All parents of invited adolescents signed a written informed consent form. According to Italian law, the respect of individual privacy was guaranteed.

Of these, 79 children (mean age 15.7 years ± 0.9; 50 males) gave their consent and, on the day of the study, each subject underwent a new skin prick test and blood sampling. Sera were tested for the presence of Par j 2 specific antibodies by means of Western blot. A non-allergic subject was enrolled as a negative control.

2.2. In silico analysis

3D modelling was performed using the SWISS MODEL Workspace (http://swissmodel.expasy.org/workspace) and the Predict Protein software (https://www.predictprotein.org). Putative antigenic determinants were analyzed by the ElliPro Prediction software (http://tools.immuneepitope.org/tools/ElliPro/iedb_input). Based on the 3D structure of a protein antigen, ElliPro predicts linear and discontinuous antibody epitopes by homology modelling. ElliPro associates each predicted epitope with a score, defined as a PI (Protrusion Index) value, averaged over epitope residues. For each residue, a PI value is defined as percentage of the protein atoms enclosed in the ellipsoid, which approximates the protein surface, at which the residue first comes to lie outside the ellipsoid; for example, all residues that are outside the 90% ellipsoid will have PI = 9 (or 0.9 in ElliPro). Prediction was performed using the 1FK1A PDB entry. Peptide similarity was studied using the Structural Database of Allergenic Proteins (SDAP) software (https://fermi.utmb.edu/).

2.3. Gene fragmentation and recombinant protein expression

Different clones were obtained by PCR amplification of the Par j2.0101 template (accession number X95865). The