Journal of Medicine and Medical Sciences Vol. 1(12) pp. 580-588 December 2010 Special issues
Available online @http://www.interesjournals.org/JMMS

Review

Allergens: sources, exposure and sensitization levels, diagnostic tools and immunotherapeutical applications

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Accepted 15 December, 2010

Allergens are proteins or glycoproteins that are usually divided in indoor allergens, such as those derived from house dust mites, molds, cockroaches and pets, and outdoor allergens, as those derived from pollen grains. Mites of the genus *Dermatophagoides* spp. are the predominant fauna in house dust worldwide. Many studies have shown that public places, such as hospitals, offices, cinemas, schools, hotels or even public and private transport vehicles may contain mite allergen levels sufficient to sensitize genetically predisposed individuals. Other important allergens in the development of allergic diseases are derived from pet and cockroaches. Can f 1 and Fel d 1 are the major allergens derived from dog and cat danders, respectively, that are investigated in allergen exposure and sensitization. Allergens from *Blattella germanica* and *Periplaneta americana* are clinically relevant in the development of asthma. Other allergen sources are derived from fungus, as *Alternaria alternata, Aspergillus fumigatus, Candida albicans, Cladosporium herbarum* and *Epicoccum nigrum*. Several species of grasses that produce pollen have been recognized as important allergen sources in temperate climates, such as *Lolium perenne, Poa pratensis, Phleum pratense, Dactylis glomerata* and *Cynodon dactylon*. For the diagnosis and immunotherapy of allergic diseases caused by these allergens, the development of molecular biology techniques was fundamental for the production, identification and characterization of several recombinant proteins. The production of hypoallergenic recombinant proteins related with allergenic sources will be important for attending the current trend to incorporate the recombinant allergens in products for the treatment of respiratory allergies, and monitoring of the immune response profile in patients under allergen-specific immunotherapy represents a vast promising field and innovative scientific research.

Keywords: Allergen exposure, recombinant allergens, hypoallergens, allergy diagnosis, immunotherapy.

INTRODUCTION

Allergic diseases are caused by immunological reactions to allergens, which are substances that induce and react with specific IgE antibodies in atopic individuals. IgE reactivity to allergens in these individuals is strongly associated with clinical evidence of immediate hypersensitivity responses (Platts-Mills and Solomon, 1993; Cromwell, 1997).

Allergens are generally proteins or glycoproteins with molecular weight (MW) above 10 kDa, but molecules with MW <1 kDa like haptens can bind to carrier molecules and induce allergic immune responses. They are commonly divided in indoor allergens, such as those derived from house dust mites, molds, cockroaches, and pets, and outdoor allergens, as those derived from pollen grains (Cromwell, 1997; Johansson et al., 2004).

According to the Subcommittee on Nomenclature of Allergens by the International Union of Immunological Societies and the World Health Organization, the name of an allergen should not be written in italic form and must be designated by the first three letters of the genus to which body belongs together with the first letter of the specie and the number corresponding to the chronological order of its discovering. For example, Blo t 5 (allergen from *Blomia tropicalis* group 5) (King et al., 1994). The current nomenclature contemplates the different molecular forms of the same allergen, called

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isoforms or isoallergens. In this case, the number of the correspondent isoform is added after the number of allergen (Blo t 5. 0101, for example) (Piboonpocanun et al., 2006).

Allergen exposure and sensitization

Sensitization to allergens depends on the individual genetic background and the allergenic exposure. Several studies have shown the importance of allergens for sensitization to house dust mite allergens, particularly in the induction of respiratory allergy. Gehring et al. (2001) demonstrated that exposure to mite allergens in house dust increased the risk of wheezing and dyspnea, whereas exposure to cat allergens was associated with nocturnal cough. Individuals exposed to high concentrations of more than one allergen had almost seven times higher risk of presenting respiratory symptoms compared with those not exposed (Gehring et al., 2001). Although fungal spores and cat allergens are important risk factors for sensitization, mite allergens may be responsible for the prevalence of asthma (Dharmage et al., 2001).

Zock et al. (2006) showed that the highest prevalence of allergens from the dust mite *Dermatophagoides pteronyssinus* occurred in the cities of Oviedo and Galdakao in Spain, where 95% of homes contained high levels of Der p 1 and more than 99% contained Der p 2. Another study conducted in Italy found high levels of allergens from dust mites in upholstered furniture located in workplace (Perfetti et al., 2004). In Brazil, studies of allergen exposure have been conducted in recent years. In the city of Ribeirão Preto, Southeastern Brazil, Tobias et al. (2004) detected high levels of group 1 allergens of house dust mite in homes of allergic patients. In addition, Baqueiro et al. (2006) demonstrated a high frequency of the house dust mite allergens *D. pteronyssinus* and *B. tropicalis* in Salvador, Bahia. Our group has demonstrated significant levels of mite allergens in house dust in homes and hotels in Uberlândia and Uberaba, Minas Gerais state (Sopelete et al., 2000; Terra et al., 2004; Simplicio et al., 2007).

Nowadays, the principal factors that are associated to increased prevalence of allergic diseases are related to (i) modern lifestyle, predominantly the sedentary life and long staying of the individuals indoors, in both homes and businesses (Pope et al., 1993), (ii) environmental and behavioral changes, such as increased temperature, decreased ventilation, use of carpets, rugs and decorative objects that retain dust, (iii) staying of children longer at home watching television or computers, (iv) increased psychological stress, (v) increased contact with pollutants, and (vi) excess of hygiene and limited exposure to microbial antigens (Ring et al., 2001; Mösges et al., 2002). In addition to the hygiene hypothesis, other factors have contributed to increased prevalence of allergic diseases in recent times, including the indiscriminate use of antibiotics and systemic steroids, which have directly influenced the composition of intestinal bacterial flora and consequently changed the profile the individual immune response (Shreiner et al., 2008).

Environments outside the home, such as transporting vehicles can also be considered important in the allergen sensitization. In Scandinavia and Finland, levels of dust allergens found in public transport vehicles were low and insufficient to cause manifestation of allergic symptoms in users, although many people have shown symptoms of respiratory allergy inside the vehicles (Partti-Pellinen et al., 2000). In a similar study performed in Uberlandia, Minas Gerais state, Brazil, we detected a higher prevalence of pet allergens in dust from cars, at levels sufficient to cause sensitization of genetically predisposed individuals (Justino et al., 2005). Curiously, the presence of pet allergens was found in car owners who did not carry their pets in vehicles. These studies indicate that transport vehicles, besides mattresses and carpets, may be considered reservoirs of mite and pet allergens, as well as important means of dispersion (Sopelete et al., 2000; Pereira et al., 2004).

Mite allergens

The house dust mite allergens induce high levels of IgE antibodies in atopic individuals, affecting more than 50% of allergic patients (Arlian et al., 2001). Mites of the genus *Dermatophagoides* spp. (Pyroglyphidae family) are the predominant fauna in house dust of the world (Arruda et al., 1994; Platts-Mills et al., 1994). The mite *D. pteronyssinus* is the most prevalent in Brazilian homes in comparison to the mite *D. farinae* (Jorge-Neto et al., 1984; Arruda et al., 1991). However, some Brazilian cities, as Uberlandia and Uberaba, Minas Gerais state, the prevalence from *D. farinae* is higher than *D. pteronyssinus* (Sopelete et al., 2000; Terra et al., 2004). In addition, other studies have shown that public places, such as hospitals, offices, cinemas, schools, hotels or even public and private transport vehicles may contain mite allergen levels sufficient to sensitize genetically predisposed individuals.

At the Second International Meeting of Dust Mites and Asthma, held in England in 1990 it was considered that exposure at levels ≥ 2 µg of group 1 and 2 allergens per gram of dust would be a risk factor for sensitization in genetically predisposed individuals, while that exposure at levels ≥ 10 µg of mite allergens per gram of dust would be a risk factor for the onset of acute asthma (Platts-Mills et al., 1992).

The major allergens from house dust mites (*D. pteronyssinus*, *D. farinae* and *B. tropicalis*) are divided into 23 groups according to the Subcommittee on Nomenclature of Allergens of the International Union of Immunological Societies and

Allergome portal. The biochemical composition, homology and molecular mass, named according to the order of discovery, and some of its main characteristics and biochemical data on the reactivity with IgE antibodies are presented in the Table 1. Among the allergen groups, several groups have enzymatic activity: (1) cysteine proteases (group 1 allergens), (2) serine proteases (group 9 allergens) and (3) amylases (group 4 allergens). These allergenic enzymes come from the gastrointestinal tract of the mites and are found in high concentrations in their feces, in particles from 10 to 40 µm in diameter, similar in size to pollen or spores. These particles are temporarily suspended in the air after "turbulence" caused by use of vacuum cleaners, or when "making the bed" (Brunton and Saphir, 1999). It is possible that proteolytic activity of many allergens promote the development of allergic inflammation. The allergen Der p 1, for example, is a cysteine protease that cleaves occludin, a protein component of compact junctions between epithelial cells of the respiratory tract. This cleavage results in the recognition of the allergen by dendritic cells, mast cells and eosinophils, with subepithelial release of proinflammatory mediators and induces IgE synthesis (Hoover and Platts-Mills et al., 1995; Wan et al., 1999). Furthermore, Der p 1 can cleave CD23 (low affinity Fc receptor for IgE, Fc epsilon RIIb) and CD25 (alpha chain of the IL-2 receptor) starting their allergenicity (Shakib et al., 1998). On the other hand, there are allergens that have not enzymatic activity, but are highly immunogenic, such as the Der p 2 allergen (Pomés, 2002). Allergens from *Blomia tropicalis*, such as Blot 5, have an important role in the sensitization of asthmatic patients living in tropical and subtropical regions, including Brazil and Florida (Fernández-Caldas, 1993; Tsai et al., 1998).

Pet allergens

Other important allergens for the development of allergic diseases are derived from pet allergens, particularly from dogs (*Canis familiaris*) and cats (*Felis domesticus*). Can f 1 and Can f 2 are the dog major allergens and are mainly distributed in the epithelial tissue of the dog tongue, even though Can f 2 is also distributed significantly in canine parotid glands. Among these allergens, Can f 1 is the most studied in both allergen exposure and sensitization. This allergen has 25 kDa and it is predominantly found in the fur, saliva and, to a lesser extent, urine and feces from dogs. Can f 2 is a lipocalin that shows 22% identity with the cat allergen Fel d 4, suggesting an important role in the co-sensitization of patients with respiratory allergy history to dog and cat allergen exposure (Madhurantakam et al., 2010). The allergen Fel d 1 is derived from fur intradermal follicles located primarily in the facial area of cats, and it is found in high concentrations in the salivary glands, tears, urine and secretions of male (Anderson and Baer, 1981; Leitermann and Ohman, 1984; De Andrade, 1996). The cat skin peeling eliminates particles of 2-5 microns in diameter containing the allergen Fel d 1, which remain in suspension for a longer period, especially in poorly ventilated environments, while maintaining their allergenic potency for over 20 weeks, even after cat removal (Hoover and Platts-Mills, 1995; Wan et al., 1999). Interestingly, allergens from pets, particularly those from cats, are more distributed in homes, schools, workplaces and public transport vehicles, where such pets have never been. This is due to the adherence of the particles that carry the allergens in clothing and shoes. Consequently, these allergens are passively transported by people who touch cats or when visiting areas where there are the gift felines (Custovic et al., 1998; Chapman and Wood, 2001). For pet allergens, previous studies have shown that values > 1 µg of allergen per gram of dust would be sufficient to sensitize genetically predisposed individuals, while values > 10 µg of Can f 1 and > 8 µg of Fel d 1 per gram of dust would be to able of inducing exacerbation of allergic symptoms in sensitized individuals (Gelber et al., 1993; Ingram et al., 1995). Recently, studies performed in homes considered as a risk factor for sensitization the exposure at levels > 10 µg/g of dust for Can f 1 allergen and > 8 µg/g of dust for Fel d 1 allergen (Tranter, 2005). However, levels considered moderate (1-10 µg/g of dust for Can f 1 and 8 µg/g of dust for Fel d 1) in alternative environments, such as schools and workplaces, are clinically relevant and may be risk for a small but significant number of individuals (Tranter, 2005).

Cockroach allergens

Two cockroach species, *Blattella germanica* and *Periplaneta americana*, very common in homes, are considered important sources of allergens in the development of allergic diseases. The Bla g 1, Bla g 2, Bla g 4, Bla g 5 and Bla g 6 allergens from *Blattella germanica*, and the Per a 1, Per a 3 and Per a 7 allergens from *Periplaneta americana* were already identified, cloned and clinically studied in the development of asthma (Arruda et al., 2010). Significant levels of these allergens are mainly accumulated in the kitchen of homes, while low levels are found in the bed, floor, room and upholstered furniture.

Fungal allergens

Allergy to fungi is not considered a typical seasonal allergy, occurring in several seasons of the year. In temperate regions, however, higher number of spores can be observed during the summer and autumn, decreasing with cooler temperatures and disappearing

Table 1. Groups of allergens from Blomia tropicalis, Dermatophagoides pteronysssinus and D. farinae according to the biochemical identity.

Group	Allergen	Isoform	MWR (kDa)	Biochemical identity	Reactivity to IgE
1	Blo t 1	Blo t 1.0101, Blo t 1.0102	26	Cysteine	62-90%
	Der p 1	Der p 1.0101-Der p 1.0124	25	protease	75-92%
	Der f 1	Der f 1.0101- Der f 1.0110	27		87%
2	Blo t 2	Blo t 2.0101-Blo t 2.0104	14	ND	63%
	Der p 2	Der p 2.0101- Der p 2.0115	14	ND	>71%
	Der f 2	Der f 2.0101- Der f 2.0117	15	NPC2 family	94%
3	Blo t 3	Blo t 3.0101	25	Trypsin	50-57%
	Der p 3	Der p 3.0101	25/28		97%
	Der f 3	Der f 3.0101	29		16%
4	Blo t 4	Blo t 4.0101	56	Alpha amilase	<15%
	Der p 4	Der p 4.0101	60		46%
5	Blo t 5	Blo t 5.0101	14	ND	42-92%
	Der p 5	Der p 5.0101, Der p 5.0102			31%
6	Blo t 6	Blo t 6.0101	25	chymotrypsin	<10%
	Der p 6	Der p 6.0101			41%
	Der f 6	Der f 6.0101			41%
7	Der p 7	Der p 7.0101	26, 30, 31	ND	46%
	Der f 7	Der f 7.0101	30/31		46%
8	Der p 8	Der p 8.0101	27	Glutathione-S-transferase	40%
9	Der p 9	Der p 9.0101, Der p 9.0102	29	Serine protease	92%
10	Blo t 10	Blo t 10.0101	33	Tropomyosin	29%
	Der p 10	Der p 10.0101- Der p 10.0103	36		5,6%
	Der f 10	Der f 10.0101	37		80,6%
11	Blo t 11	Blo t 11.0101	110	Paramyosin	12-52%
	Der p 11	Der p 11.0101	103		42-67%
	Der f 11	Der f 11.0101	98		87,6%
12	Blo t 12	Blo t 12.0101	14	ND	50%
13	Blo t 13	Blo t 13.0101	15	fat acid binding	11%
	Der f 13	Der f 13.0101	ND	protein	ND
14	Der p 14	Der p 14.0101	177	Apolipoforin	ND
	Der f 14	Der f 14.0101			65,8%
15	Der f 15	Der f 15.0101	98/109	Chitinase	70%
16	Der f 16	Der f 16.0101	53	Gelsoline/Villi	47%
17	Der f 17	Der f 17.0101	53	Calcium binding protein	35%
18	Der f 18	Der f 18.0101	60	chitinase	54%
19	Blo t 19	Blo t 19.0101	7.2	Anti-microbial peptide	3%
20	Der p 20	Der p 20.0101	ND	Arginine chitinasee	Baixo
21	Blo t 21	Blo t 21.0101	15	ND	93%
	Der p 21	Der p 21.0101	14		ND
22	Der f 22	Der p 22.0101	ND	ND	ND
23	Der p 23	Der p 23.0101	14	ND	ND

Adapted: Subcommittee on Nomenclature of allergens IUIS and Allergome. ND, not determined.

completely in the presence of snow. The main hosting sites of fungal spores are the carpet, wallpaper and heating systems or air conditioners. Currently, the main species of allergologic relevance are *Alternaria alternata, Aspergillus fumigatus, Candida albicans, Cladosporium herbarum and Epicoccum nigrum*, which show considerable variation in their biochemical composition. Among the major allergens of *A. alternata*, the Alt a 1 (25 to 50 kDa) glycoprotein with at least five isoforms, and Alt a 2 (6 kDa), which showed to be reactive in more than 80% of allergic patients are described (Unger et al., 1999). *Aspergillus* sp spores were the first to be identified as allergenic sources, and are causal agents of many other diseases. The major allergen identified in *A. fumigatus* was Asp f 1 (18 kDa), which has considerable homology with mitogillin, a potent cytotoxin (Arruda et al., 1990). The yeast *C. albicans* is the most frequently isolated species of pathogenic material in its genus,

though it is found predominantly as body flora of the gastrointestinal tract. The IgE-mediated response is predominantly directed against two proteins: one of 29 kDa and another of 46 kDa (Savolainen et al., 1989). The more abundant fungal spores in the world, especially in temperate regions, are from *Cladosporium spp*, especially *C. herbarum*. The two major allergens of this species are the glycoproteins Cla h 1 (13 kDa) and Cla h 2 (20-22 kDa) (Aukrust and Borch, 1979). Another important fungal specie is *E. nigrum*, which has been shown to be reactive in enzyme linked-immunosorbent assays (ELISA) and skin prick test (SPT) in 20 to 30% of atopic patients (Portnoy et al., 1987). A study involving children in an urban low-income community in Recife, Northerneast Brazil, identified a high frequency (92%) of hypersensitivity to at least one extract of fungus examined (Osório et al., 2006). In Porto Alegre, Southern Brazil, a study revealed high occurrence of fungal spores in the air outside the home, since they are responsible for 15.4% of allergic sensitization in atopic individuals with asthma and/or rhinitis (Mezzari et al., 2003).

Pollen allergens

The sensitization to pollen allergens may occur isolated or associated with sensitization to other perennial allergens, such as allergens from house dust mite, fungi and pet epithelium. Thus, the symptoms may occur exclusively during the spring, the pollen season, or throughout the year, but in the latter case, exacerbated in the spring. In Brazil the symptoms usually began in September and are intensely exacerbated in the months of October and November, extending in some cases even December/January (Vieira, 1995). Several species of grasses that produce pollen have been recognized as allergen sources, including *Lolium perenne, Poa pratensis, Phleum pratense, Dactylis glomerata* and *Cynodon dactylon* (Suphioglu, 2000; Weber, 2003). *Lolium perenne* and some grasses in the subfamily Pooideae are important sources of allergens in temperate climates, while pollen grains of *C. dactylon* are important in tropical climates (Freidhoff et al., 1986; Wüthrich et al., 1995; Schumacher et al., 1985). The *Phleum pratense* pollen is an important allergen source in temperate regions such as Northern and Central Europe (Petersen et al., 2006). A clinical trial performed with recombinant allergens of grasses indicated that four major allergens from grass pollen *Phleum pratense* (Phl p 1, Phl p 2, Phl p 5 and Phl p 6) cover the most relevant epitopes needed for diagnosis and treatment of grass pollen allergy (Jutel et al., 2005). Overall, eleven groups of allergens have been described in one or more species of grasses (Suphioglu, 2000). These groups represent a variety of glycosylated and non-glycosylated proteins in size, structure and physicochemical properties (Anderson and Lidholm, 2003). From a clinical viewpoint, group 1 allergens (27-35 kDa) are the most important and recognized by approximately 95% of all patients sensitive to grass pollen, followed by group 5 allergens (27-38 kDa) that are recognized up to 85% of these patients (Weber, 2003). Other important allergen groups are the groups 2, 3, 4 and 13 that are recognized by more than 50% of allergic individuals (Suck et al., 2000).

In Brazil, the grass pollen contributes nearly all cases of pollen illness, and allergens from pollen grains of trees and herbs would have less importance in the induction of sensitization and pollinosis in atopic patients compared with grasses (Vieira, 2003). Four tree species of the Southern Brazil as *Platanus sp, Ligustrum sp, Acacia sp, Araucaria sp* and *Eucalyptus sp* are able to dispense large amounts of strongly allergenic pollen to the environment (Vieira, 2003). Other allergenic grass species as *Anthoxanthum odoratum* (sweet grass), *Cynodon dactylon* (bermuda grass), *Holcus lanatus* (woolly grass), *Paspalum notatum* (fork grass) and *Bromus sp* grow wildly in the periphery of towns and uninhabited lands (Vieira, 2003; Kurtz 1998). In Brazil, *Lolium multiflorum* (rye grass or Italian ryegrass) is a grass with high allergenic potential and considered the main cause of grass pollinosis, especially in Southern Brazil. It is winter non-native forage that was brought to Brazil by European immigrants to be used in agriculture. From the ecological viewpoint *L. multiflorum* spreads and grows wildly in non-agricultural areas along highways, railways, transmission lines, uninhabited lands in cities and even on sidewalks and roads (Dutra et al., 2001). Thus, densely populated cities may have ryegrass pollens being transported by wind at the time of pollination (Vieira, 2003).

Few studies have been developed to identify the major allergenic fractions present in the pollen extract of *L. multiflorum* that are recognized by IgE antibodies in patients with pollinosis (Sopelete et al., 2006; Bernardes et al., 2010). Thus, studies that characterize IgE, IgG1 and IgG4 responses to the major allergens of *L. multiflorum* and their relation to pollen allergens of other grasses are very interesting and are being performed by our research group to elucidate the immune response to grass allergens. Also, studies that characterize *L. multiflorum* isoforms and its major allergens are scarce in the scientific literature, with only one allergen described, Lol m 5, with molecular mass of 27-38 kDa (Mohapatra et al., 2005). In a study using monoclonal antibody to Phl p 5, group 5 allergens were detected in some grass extracts, including *L. multiflorum* extract (Schäppi et al., 1999). In our recent work, we evaluated the immunodominant allergenic fractions in the *L. multiflorum* pollen extract that are recognized by IgE antibodies in patients with pollinosis, demonstrating that fractions of 28-30 kDa and 31-34 kDa are recognized by more than 90% of sera from these patients living in Southern Brazil (Sopelete et al., 2006).

Food allergens

The frequency and variety of illnesses caused by allergen exposure from the diet may be controversial, although IgE-mediated immune mechanisms occur from eating foods, such as cow milk, peanuts, shellfish and fruit. The antigens present in these foods can induce both type I and type IV hypersensitivity responses and the release of inflammatory mediators can be due to proinflammatory properties intrinsic to food allergens. The ingestion of food allergens causes a variety of clinical syndromes, including anaphylaxis and neurological, gastrointestinal, skin and respiratory disorders.

The diagnosis of food allergy can be difficult if there is no clear evidence of IgE reactivity to food allergens. Usually, allergens from birds, fish and mammals belong to the family of parvalbumin, tropomyosin, and lactalbumin profilins, whereas allergens from plants are related to prolamin, cupin and pathogenesis-related proteins.

There are many house dust mite and cockroache proteins with high cross-reactivity with tropomyosin from shrimp, lobsters, crabs, squid, clams and snails or paramyosin from *Schistosoma mansoni* and *Onchocerca volvulus*. Therefore, cross-reactivity between allergens from different sources may be relevant in the development of allergic reactions.

Allergen injection

Allergen exposure by via injectable is associated with certain constituents of saliva or insects, or injecting drugs. Allergy to venom of Hymenoptera (bees, wasps and ants) represents a potentially serious problem for affected individuals, and could cause, since local reactions to systemic toxic manifestations and anaphylactic reactions. In Brazil, bees as *Apis mellifera* [Api m 1(phospholipase A2) and Api m 2 (hyaluronidase)] and ants as *Solenopsis invicta* [Sol i 1 (phospholipase A and B), Sol i 3 (component of the antigen 5 family) and Sol i 4 (8 to 10% of the total concentration of poison)] are the main insects whose venoms present considerable allergenic proteins (Yang and Castro, 2007).

Reactions to drugs are frequents in the clinic practice (Lazarou et al., 1998). Some drugs induce reactions whose symptoms are similar to IgE-mediated reactions (Bernd, 2005). The main drugs that induce IgE-mediated responses are amoxicillin, ampicillin, cefaclor, bovine or human insulin, penicillin G and A, protamine, and tetanus toxoid. Allergic reactions to these drugs may manifest as rash or anaphylaxis. The frequency of sensitization to these drugs as antibiotics can vary from 1 to 10% in the general population.

Techniques for studying allergen exposure, sensitization and immunodiagnostic

The quantification of major allergens from house dust was feasible with the development of immunoenzymatic assays (ELISA). The use of a simple and sensitive ELISA using two monoclonal antibodies (sandwich ELISA) for specific allergens has routinely allowed the determination of allergen levels in dust samples (Luczynska et al., 1989).

Sensitization to the most common indoor allergens can be assessed by skin reactivity tests or assays that determine the serum levels of specific IgE to allergens (Platts-Mills, 1996). The diagnosis of allergy by *in vivo* tests (skin prick or intradermal) is useful for identifying patients with IgE-mediated hypersensitivity. They are tests with high specificity and sensitivity, fast and simple to perform, and are economical tests when compared to the laboratory determination of specific IgE antibodies. However, the skin test does not diagnose the allergic disease. It simply determines the presence or absence of allergen-specific IgE antibodies, which are important in the allergy pathogenesis (OWBY, 1988).

One of the most used *in vitro* methods for determination of specific IgE is indirect ELISA. However, in research laboratories, the use of the technique Immunoblot is required to identify the major allergenic fractions present in total extracts.

The use of advanced methodologies such as two-dimensional electrophoresis and immunoblot may contribute to the identification and characterization of a broad repertoire of allergen isoforms and their relevance in the context of allergic diseases. These isoforms may have different antigens that are recognized by sera of allergic patients, thus enabling the use of such allergens in serological techniques that detect and measure specific antibodies in patients with respiratory allergy, and also in the laboratory monitoring of patients who are under allergen-specific immunotherapy. Therefore, the use of *in vivo* and *in vitro* tests for determination of specific IgE antibodies to allergens have contributed to the evaluation of sensitization in atopic and non atopic subjects.

Recombinant allergens and hypoallergens *versus* allergy diagnosis and immunotherapy

Despite the development of natural allergenic products for skin tests with high quality standard over the past 20 years, they are still heterogeneous and contain various non-allergenic proteins and other non-active components, and in some cases, endotoxins. Moreover, the risk of contamination by allergens from other sources can mask

the allergenicity of certain proteins (Chapman et al., 2000). Thus, the development of molecular biology techniques has allowed the production, identification and characterization of several recombinant proteins in large scale, which have importance in the diagnostic sensitivity and specificity, as well as in the more detailed study of factors affecting the allergenicity and immunogenicity of a particular allergen. A careful selection of allergens to be cloned in recombinant allergens can result in allergenic activity compared to that displayed by natural allergens (Scheiner and Kraft, 1995; Valenta et al., 1999).

The use of recombinant allergens for diagnostic purposes is justified by the possibility of systematic production and high purity of the same, which minimizes the risk of adverse and unpredictable reactions in skin tests, and is more sensitive for diagnostic tests (Chapman et al., 2000).

Clinical studies have demonstrated the efficacy of specific immunotherapy with administration of increasing doses of crude extract of mite allergens through the induction of tolerance of peripheral T cells, an essential step forward in the normal immune response to allergens (Larché, 2006; Karamloo et al., 2005). However, the risk of inducing anaphylactic reactions may be significant for many patients. Thus, to minimize adverse effects resulting from immunotherapy with crude allergen extracts, some studies have shown the importance of using hypoallergenic immunotherapy in allergic diseases (Niederberger et al., 2004).

To reduce the allergenic activity of a protein, it has to be changed so that there may be little or no reactivity with IgE. However, the epitopes for T cell recognition must be preserved so that it evokes protective immune response with reduced risk for anaphylactic reactions (Niederberger et al., 2004). Therefore, new strategies have been tested for the study and characterization of regions with IgE-binding capacity and conformations that mimic epitopes, called mimetopes (Shakib et al., 2008).

The mimetopes constitute a region of the allergen that is recognized by IgE antibodies, thus representing the structure of an epitope on B cells. Therefore, they can be employed to develop a new strategy for immunotherapy of peptides as vaccines or specific treatments with hypoallergenic protein. So, mimetopes could be used for the induction of IgG antibodies to block the interaction of allergens with IgE antibodies (Shakib et al., 2008).

The identification and characterization of epitopes capable of generating an immune response against the mites enable the use of hypoallergenic proteins, recombinant or chemically synthesized peptides in vaccines in industrial scale. This approach works only the immunogenic region, rendering unnecessary the use of the complete protein, or when using the complete protein it should present minimal or even depleted the epitopes responsible for the unwanted responses. As shown, a vaccine based on the selection of specific epitopes comprising different antigens from different classes is a very viable possibility in the formation of a poly-immunogenic vaccine compared to other traditional methodologies in use, with the advantage of selecting desirable characteristics, such as high purity, known chemical characterization, absence of contaminants, large-scale production, easy storage due to high stability, absence of proteolytic enzymes, and low cost industrial scale production (Naik et al., 2008; Carnés and Robinson, 2008).

Thus, production of hypoallergenic recombinant proteins related with allergenic sources will be important for attending the current trend to incorporate the recombinant allergens in products for the treatment of respiratory allergy, and following the response profile in patients under allergen-specific immunotherapy represents a vast promising field and innovative scientific research.

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