

REVIEW ARTICLE

Allergy to House Dust Mites and Asthma

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House dust mites have been shown to be important sources of indoor allergens associated with asthma and other allergic conditions. Asthma is a chronic respiratory disease that affects millions of people worldwide, and numerous scientific studies have shown that the prevalence of asthma is increasing. The most common dust mite species around the world include *Dermatophagoides pteronyssinus* (Dp), *Dermatophagoides farinae* (Df), *Euroglyphus maynei* (Em) and *Blomia tropicalis* (Bt). Over the past three decades, many important allergens from these species have been identified and characterized at the molecular level. The biological function of several house dust mite allergens has been elucidated, with many of them showing enzymatic activity. However, Bt allergens remain the least studied, even though this mite is very common in tropical and subtropical regions of the world, including Puerto Rico. Therefore, it is very important to include Bt in diagnostic and therapeutic strategies for house

dust mite induced allergy and asthma, particularly in areas where Bt exposure and sensitization is high. Recombinant DNA technology, as well as other molecular biology and immunological techniques, have played a fundamental role in advances towards a better understanding of the biology of house dust mites and their role in allergic diseases. This kind of study also contributes to the understanding of the complex immunologic mechanisms involved in allergic reactions. The development of effective diagnostic and therapeutic approaches depends on the continuity of research of house dust mite allergens. The objectives of this review are to describe the most important aspects of house dust mite allergy and to acquaint the scientific community with the latest findings pertaining to house dust mite allergens, particularly those derived from Bt.

Key words: Allergy, Asthma, House dust, Mite, Blomia tropicalis, Mite allergens

Allergy is an exaggerated or hypersensitivity reaction of the immune system to specific external substances called allergens, which are usually harmless to most people. Common causes of allergy include animal dander, house dust mites, foods, pollen, insects, and chemical substances. Allergy to house dust mites is a condition that affects millions of people around the world, including Puerto Rico and some areas of the United States. Some common conditions caused by allergy to house dust mites include asthma, allergic rhinitis and conjunctivitis. Although allergy to house dust mites is not a direct threat to a person's life, asthma could become a serious condition that, if not properly treated, could lead to death due to respiratory complications. Asthma is a chronic respiratory disease, often of allergic origin, that is

characterized by continuous or paroxysmal labored breathing accompanied by wheezing, bronchial constriction and inflammation, and often by attacks of coughing or gasping. The prevalence of asthma in the United States and other countries has been increasing during the last ten years (1-3). Diverse studies suggest that exposure to house dust mite allergens may be a primary cause or a risk factor in the development of asthma, and that it can act as a trigger for the exacerbation of the symptoms (4-8). A 2002 study by Terreehorst and collaborators with 325 atopic patients showed that 92% of the asthmatic patients and 85% of the patients with atopic dermatitis had a history of allergic rhinitis, and that there was a high prevalence of nasal symptoms associated with sensitization to house dust mites in these patients. These investigators conclude in their study that asthma and atopic dermatitis are highly associated with allergic rhinitis, and that allergic rhinitis is a risk factor for asthma (9). Other epidemiology surveys have reported similar results (10). The American Academy of Allergy, Asthma and Immunology reports up to 78% of asthmatic patients with nasal symptom and up to 38% of allergic rhinitis patients with asthma (11). Allergic rhinitis is the inflammation of

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the mucous membrane of the nose as a result of exposure to an allergen. It is a very common condition not as serious as asthma, but very debilitating and with a negative impact in the quality of life of the affected person. Allergic rhinitis caused by house dust mites is perennial, with symptoms appearing continuously or intermittently all throughout the year. People with allergic rhinitis or asthma often have a family or personal history of atopy. Atopy is the increased tendency, with a genetic origin, to produce immediate hypersensitivity reactions usually mediated by immunoglobulin E (IgE) antibodies against normally harmless substances. Diverse scientific studies propose the atopy concept as the basis for a connection among allergic conditions such as atopic dermatitis, allergic rhinitis, asthma and conjunctivitis (9). Allergic conjunctivitis is the inflammation of the conjunctive membrane of the eye caused by airborne allergens that invade it, and atopic dermatitis is the inflammation of the skin as a result of a hypersensitive reaction.

The onset of allergic rhinitis and asthma usually occur during childhood, adolescence or early adulthood. Symptoms may decrease in older people, but more often persist throughout their lifetime (11). Allergic and asthmatic people have to deal with their conditions in a daily basis, seeing their energy and productivity levels negatively affected and, therefore, their overall daily performance as well. Moreover, these conditions require scheduling frequent visits to their allergists and doctors, often having to take time from school and work. Allergic rhinitis and asthma may also influence adversely over other common activities such as sleeping, learning and social interactions. Therefore, not only is the physical state of the person adversely affected but the emotional well-being as well.

The development of allergy starts when the individual is in contact with the allergen for the first time and becomes hypersensitive to it. When the allergen invades the mucous membrane of the nasal passages an immediate allergic response occurs. The most accepted mechanism indicates that the allergen is presented to T helper-2 (T_H2) lymphocytes by antigen presenting cells (APC), and the T_H2 cells respond by releasing cytokines such as interleukin-4 (IL-4) and interleukin-5 (IL-5) which attract inflammatory cells to the airways, such as the mast cells, basophils, and eosinophils. T_H2 cells activate B cells of the immune system, which produce a specific antibody, IgE, which is overproduced in subsequent allergic reactions. IgE antibodies attach to membrane receptors in mucosal mast cells and activate them. The allergen binds those antibody molecules causing the release of mast cell granules to the outside of the cell. These granules contain chemical inflammatory substances such as histamine and

leukotrienes, which trigger the array of symptoms that comprise the allergic reaction. At the same time, mast cells secrete IL-4, which stimulates B cells to produce more IgE. In chronic allergic asthma the allergen induces activation of submucosal mast cells in the lower air passages, leading to bronchial constriction and an increase in fluid and mucus secretions which make breathing more difficult. These phenomena are a result of a late-phase reaction, characterized by the continuous synthesis and release of chemical mediators such as cytokines and leukotrienes from the activated mast cells and eosinophils. Mast cells also secrete IL-5, which further stimulates eosinophils for their release of more inflammatory mediators. In chronic allergic rhinitis occurs a similar late-phase reaction (11-14).

It is not well known what structural features or what functional properties of allergens confer them the capacity to modulate the immune response toward the T_H2 cell activation, which stimulates an IgE response (15, 16). However, although much research is still necessary, it has been observed that many common allergens share typical characteristics such as enzymatic activity, relatively low molecular weight (less than 60-70 kDa) and high solubility. Moreover, it has also been shown that factors extrinsic to the allergen are involved, such as genetic predisposition to overproduce IgE (atopy) and the particular immune response of the individual, the nature or degree of exposure to the allergen and other environmental factors (2, 16, 17).

The most common symptoms of allergic rhinitis and allergic asthma caused by inhaled house dust mite allergens include sneezing, itchy eyes, ears and nose, local edema, nasal secretions, nasal congestion, itchy palate and throat, cough, shortness of breathing, and wheezing. Allergy to house dust mites can be diagnosed in several ways. Probably the most common method is a skin-prick test, where the patient is pricked usually in the upper arm with small amounts of different allergen extracts to evaluate the reaction to them. Allergen extracts have been traditionally prepared from natural materials and, although the quality of natural allergen extracts has improved much during the past two decades, there are several disadvantages associated to them. First, they may contain nonallergenic proteins and other molecules, such as proteolytic enzymes which may cause degradation and loss of potency in the extract. Second, they may vary in allergen composition and content. Third, they may be contaminated with allergens from other sources (18). This scenario is where recombinant allergens come to play a role making it possible to design diagnostic tests which are more allergen-specific and therefore, more tailored to the patient's allergic profile. Another diagnostic approach is measuring the levels of IgE antibodies specific for a

particular allergen through tests such as Enzyme-Linked Immunosorbent Assay (ELISA) or Radioallergosorbent test (RAST). In ELISA the IgE binding is detected by a colored reaction product while in RAST a radioactively labeled allergen is used.

Many scientists agree that the prevalence of house dust mite allergy and asthma has been increasing worldwide during the last decades. Although there is controversy regarding the causes for this increase, it is generally believed that the causes might be among factors in the environment (19). It is often proposed that these factors probably have to do with changes in lifestyles and housing in developed countries, particularly in Western societies. For example, exposure to house dust mite allergens may be increasing due to spending more time indoors, more insulation and central heating, reduced ventilation for energy saving, and carpeting in homes, making them an ideal habitats for dust mites (2, 4, 8, 11, 20). A study of 1993 in southern Puerto Rico showed that there was a significant association between hospitalizations due to asthma exacerbations and an increased precipitation and decreased wind velocity (21). This finding lead the investigators to conclude that asthmatics living in tropical islands such as Puerto Rico might be affected by an additive effect of pollutants transported in air currents. Environmental, genetic and socio-psychological factors may be interrelated and involved in the immune response to allergens, but conclusive knowledge in these areas is still under scientific scrutiny. Therefore, it is imperative to continue researching to understand the biology of house dust mites and their allergens, the immune response to them, and to find better strategies toward the control of these microorganisms and the treatment of the conditions they cause.

House Dust Mites

House dust mites belong to the phylum Arthropoda (i.e., animals with external skeletons and jointed limbs), subphylum Chelicerata, class Arachnida, order Acari, and sub-order Astigmata (lacking specialized respiratory organs) (22, 23). Contrary to what some people might believe, house dust mites are not closely related to insects, which belong to subphylum Uniramia. The morphology and physiology of house dust mites differ greatly from those of insects. For this reason, common insecticides used successfully to kill insects are ineffective for controlling house dust mite populations (23). These microorganisms usually measure between 0.1 to 0.6 mm, so they are not visible to the unaided eye, for proper identification at least 10X magnification lens are necessary. Their bodies are oval-shaped and creamy to translucent

white, and they have eight legs. The reproduction of house dust mites is sexual, with mating of a male and a female. The life cycle of house dust mites starts with the fertilized female laying a couple of eggs per day. Six-legged larvae hatch from the eggs and remain active for some time, then shed their integuments and become eight-legged resting protonymphs. The protonymphs also shed their integuments and become larger active tritonymphs. Finally, the tritonymphs undergo another shedding of skin developing into active adult mites (24-27). The period of time of this developmental cycle is uncertain, fluctuating from 2 to 6 weeks. The number of eggs a female may lay is also unclear, but it is estimated between 40 to 100 eggs over a six-week lifespan. Adult house dust mites may live between 2 to 5 months, depending on the environmental conditions. These microorganisms feed mainly from skin scales shed by humans and their pets, which are colonized by fungi, yeasts and bacteria, although they may also take advantage of other organic detritus that accumulates in homes (4, 10). Competing or predated interactions between house dust mites and fungi are not clearly defined, but it was traditionally thought that fungi enhance rates of mite population increase (27). Therefore, an approach considering fungi as a biotic factor for the control of house dust mites seems of little relevance yet. However, more research is necessary in this area and in the possibility of competitive interactions among different species of house dust mites. Proteins found in the metabolic waste products excreted in the feces by these microorganisms are the cause of the allergic reaction to them. A floating dust cloud seen in the light when dealing with bed clothing and similar materials may contain such waste products.

House dust mites inhabit areas and items of the house, as well as the workplace, that comply with their survival requirements, such as carpets, curtains, mattresses, pillows, soft toys, books, and other pieces of upholstered furniture. Common house dust mites such as *Blomia tropicalis* (Bt) and *Dermatophagoides pteronyssinus* (Dp) are inevitably found in every household, predominantly in areas of the world with high relative humidity (>45%) and warm temperatures, between 65 to 85°F (27). House dust mites satisfy their requirement of water by taking up vapor from the surrounding air and that is why many scientists suggest maintaining a low humidity indoors as a measure to reduce the allergen levels in houses. A study in India revealed that the population of mites during the summer was lower than the rainy season of September to October and the winter (28). In this study, the investigators concluded that the extremely high temperatures of the summer season, the low relative humidity (around 25%) and the lack of rain were unfavorable environmental conditions for the growth and thriving of mites. They

also found that the number of house dust mites present in the beds was higher than that of floor dust in most of the homes, concluding that it is probably due to the association of these mites with the human habitat. Moreover, they observed that old and humid houses with poor ventilation were more favorable for the survival of house dust mites in comparison with newer and well ventilated houses. These findings of seasonal variation, house dust mite concentrations and type of dwelling were directly proportional to the severity of allergic attacks in different groups of allergic and asthmatic patients studied simultaneously. In another study of 1997 with asthmatic patients sensitized to house dust mites, the investigators found that changes in airway hyperresponsiveness and other immunological parameters are associated with allergen exposure and seasonal variations (29). They found that there was a tendency to higher concentrations of house dust mite allergens during autumn than in spring. In correlation with this finding was the interesting observation that in the sensitized patients the airway hyperresponsiveness and the concentrations of serum total IgE and house dust mite specific IgE were also higher in autumn. Humidity was also higher during autumn compared with spring.

Certain measures can be followed to control these microorganisms at home. For example, washing fabrics at least weekly in very hot water and using acaricides can kill dust mites. Frequent dry and wet vacuum cleaning helps, although most allergist will recommend getting rid of carpets as well as other similar home items. Putting special covers to mattresses and pillows and using mechanical ventilation systems or air conditioning in the house are also recommended measures. Maintaining the relative humidity below 50% is a key factor for reducing dust mites levels (23, 25). However, these and other meticulous control practices must be followed constantly and for indefinite time, an extremely difficult task for most people. Special mattress covers, acaricides, as well as other anti-allergen products to minimize contact with the dust mites can be expensive and not easily found in stores. These control methods should be used in combination and can have different effects in different homes. For example, the effectiveness of acaricides will depend on method of application, type of carpets and furniture, amount of dust in the house, and quality of post-treatment vacuum cleaning (10). Besides, control of these microscopic creatures has proven to be very difficult because they reproduce themselves relatively rapidly. In the other hand, scientific studies have shown that asthmatic patients who are transferred to high altitudes or mite-free dwellings experienced reduced symptoms such as bronchial hyperreactivity. Therefore, some scientists support dust

mite avoidance measures as an effective alternative for controlling allergic diseases (4, 29).

Blomia tropicalis

Blomia tropicalis (Bt) belongs to the Glycyphagidae family. House dust mites in this family are characterized by numerous long dorsal setae (bristles), no dorsal shield, and no anal suckers. Their bodies are covered by minute papillae (26, 30). Members of the genus *Blomia* may be differentiated from other genera in this family because their legs lack a sub-tarsal scale present in the others, and also they have no claw. In addition, there are other subtle morphological differences among the species in this genus that allow differentiation of Bt from the other. These species are usually found in areas with very humid climate, such as the tropics and subtropics, including the island of Puerto Rico, although some of the members may be found in temperate regions (25, 26, 31-33). These mites have been traditionally referred to as “storage mites” or “stored-products mites” because they have been found mainly in stored grain and flour, barns, hay and straw (5, 22, 34). In the beginnings of scientific research with storage mites they were mostly associated with occupational allergic disease. Nevertheless, Bt and other Glycyphagid mites are also commonly found in homes nowadays, in rural and urban areas and therefore, are included in the “house dust mites” or “domestic mites” group. The biology and ecology of Bt are the least studied among the major house dust mites. However, it has been found in several studies to be an important source of indoor allergens, mostly in the Southern hemisphere (33). Glycyphagid mites and most other common house dust mites have been also found in other habitats such as mammal and bird nests (35). This observation is no surprise considering that human habitats share certain characteristics such as heat, moisture and food availability with these other habitats that make them an ideal dwelling for mites. In a 1997 study in Puerto Rico, Bt was found as the second most common house dust mite with 31.6%, behind *Dermatophagoides pteronyssinus* with 45.6% (31).

***Dermatophagoides* and other Species**

Dermatophagoides species of house dust mites belong to the family Pyroglyphidae and are the predominant ones in temperate and tropical regions of the world, including North America and Latin America (5). This genus is the most studied as it is evident in the scientific literature. Members of the Pyroglyphidae family are characterized by the presence of anal suckers, an anterior dorsal shield,

and body with “fingerprint” pattern of striations and setae of variable length (26). The term “house dust mites” has been traditionally used to include mainly members of the Pyroglyphidae family that live permanently and almost exclusively in house dust. However, in the Second International Workshop on Mite Allergens and Asthma (1990) it was recommended to use the term “domestic mites” to include this family and also the Glycyphagidae family, to which Bt belongs (5). Among the members of the Pyroglyphidae family, the most common are *Dermatophagoides pteronyssinus* (Dp) and *Dermatophagoides farinae* (Df), both with worldwide distribution. From the 49 species of Pyroglyphidae dust mites, 13 have been found in house dust while the others live mainly in bird nests or feathers (35). In Europe, Japan, North America, New Zealand and Australia the predominant Pyroglyphidae genera are *Dermatophagoides* and *Euroglyphus*, which are closely related (4, 34). In fact, Dp is the most commonly dust mite found in homes in Europe, followed by Df and *Euroglyphus maynei* (Em) (34-36). Em has also been found in Korea, China, India, New Guinea, and Southern United States. In Puerto Rico, Dp, Df and Em are the most common species of house dust mites, along with Bt from the Glycyphagidae family (31, 32). Another species in this family that is being studied is *Dermatophagoides siboney*, which has been found also in tropical environments, including Cuba and Puerto Rico (31, 37, 38).

Treatment of House Dust Mite Allergy

During the past ten to fifteen years, scientists have been investigating new approaches to the treatment of house dust mite allergy. A variety of medications for minimizing allergy symptoms are currently available. However, these medications have to be taken indefinitely and many of them can cause side effects such as drowsiness, which affects the patient’s daily performance. Other more effective and non-drowsy medications have to be prescribed and can also be very expensive. Control and avoidance measures at home have to be followed aggressively, and implementation of these practices can be very difficult. Immunotherapy, or hyposensitization, with dust mite allergens is another common preventive therapeutic approach. The patient is injected over a period of 1-4 years with increasing doses of the mite extract until reaching a maintenance dose, starting once a week and reducing the frequency to one or two injections a month depending on the patient’s response (24, 39). The aim of this procedure is to gradually induce tolerance to the mite allergens in the patient’s immune system. Scientific studies have shown that immunotherapy is an effective treatment, but also

indicate that the precise mechanisms involved in immunotherapy are still unclear. Some studies have shown that after the immunotherapy there was a down-regulation in the overall allergen-specific production of inflammatory cytokines such as IL-4, IL-5 and interferon-gamma (IFN- γ) (39, 40). Proposed mechanisms include the switching of the allergic immune reactivity from a T_H2-type response to a T_H1-type (i.e. immune deviation), peripheral T cell unresponsiveness (anergy), or possibly deletion of allergen-reactive lymphocytes. Immunotherapy can be expensive, but it has the advantage of being aimed to the cause of the allergy and not only the symptoms, and thereby to eliminate or reduce dramatically the allergic reaction. It has been documented through extensive studies that immunotherapy may be one of the best therapeutic strategies for dust mite allergy. However, it has been observed that the use of dust mite extracts may involve some disadvantages. One of them is the possibility of anaphylaxis (severe life-threatening allergic reaction), although this is apparently minimized with the application of very small doses over a long period. Additionally, in mite extracts it is difficult to standardize mixtures of allergenic and nonallergenic components, including proteins, carbohydrates and nucleic acids (41, 42). The quality of the extract is influenced by several factors such as the extraction procedure and storage conditions. Certain allergens may not be well represented or may even be degraded during the preparation of the mite extract (43). Consequently, the use of extracts allows the identification of the source of the allergen but not the specific molecule to which the patient is allergic to, nor the determination of IgE levels against a particular allergen. Current diagnosis and treatment of dust mite allergy is mainly based on the use of crude mite extracts. A more effective approach would be to make a diagnosis and design a therapy according to the patient’s allergen reactivity profile. This approach would allow the identification of the specific mite allergenic components causing the disease and the measurement of IgE levels against them. It is in this scenario that the design and availability of recombinant allergens play a fundamental role.

House Dust Mite Allergens

The Allergen Nomenclature Sub-Committee, International Union of Immunological Societies (World Health Organization) establishes guidelines for the identification of a molecule as an allergen (44). Only allergens with a frequency of IgE reactivity above 5% will be included in the nomenclature. Also, an allergen may be classified as a “major” or a “minor” allergen depending on whether more or less than 50% of patients tested reacted

with the corresponding allergen-specific IgE in the given system. Of course, there are inherent factors that impose some uncertainty to the determination of IgE prevalence such as choice of test system, criteria for selection of patients, geographic region, environmental conditions and others. Therefore, researchers are encouraged to perform their analysis with a substantial number of patients whenever possible. The Sub-Committee recommends analyzing the defined component for allergenic activity with at least 20-30 human sera from highly allergic individuals (45).

During the past three decades it has been well demonstrated by different scientific investigations that allergens from house dust mites constitute a major etiologic factor of allergies and asthma in many countries around the world (4, 5, 7, 46). Extensive studies have been conducted in search of a better understanding of the biological, chemical and structural properties of dust mite allergens as well as other factors that might be influencing or determining their allergenicity. Thanks to advances in molecular biology technology, the biological function and structural properties of many allergens have been elucidated, although many investigations with allergens are still in progress or are yet to be undertaken. Although the biological function of allergens has not been shown to be the only or the main responsible factor for their allergenicity, it may facilitate the immunological milieu required for specific sensitization toward an allergen or enhance the ability of the protein to trigger an IgE antibody response. For example, scientists have observed that allergenic molecules with enzymatic activity, such as cysteine proteases, irritate the mucosal surface facilitating their own processing (15, 18). The best characterized allergens of house dust mites are those in group 1, which have been identified as cysteine proteases. One of the most studied allergens from this group, Der p 1, seems to enhance allergenicity by several mechanisms such as increasing the permeability of the respiratory mucosa, enhancing antigen processing, promoting IgE synthesis, and augmenting T_H2 cell responses (15, 18, 47). Der p 1 was shown to cleave the low affinity receptor for IgE, CD23, and this action seems to disrupt the negative feedback regulation of IgE synthesis mediated by this receptor (48). However, enzyme function is not essential to trigger IgE responses, as other types of biological functions have been found for several allergens from house dust mites and other sources (7, 10, 18). Other proteins from house dust mites that have shown to be allergenic include group 3 allergens which are serine proteases, group 4 (Der p 4) with amylase activity, group 6 allergens (Der p 6) identified as chymotrypsin-like proteases, group 10 as a tropomyosin (a structural protein),

group 13 as a fatty acid binding protein, and allergens from the groups 2, 5, 7, and 12, all with unknown biological function (7, 18, 49-51). The sensitizing dose of an allergen has been debated in several studies, but in the Indoor Allergens and Asthma: Report of the Third International Workshop it was established as 2 mg allergen/g of dust (100 mites/g) (7).

The structural stability of allergens may also play an important role in the allergic response toward them, as has been shown in some studies where IgE epitopes have been altered or the three-dimensional structure of the protein has been split (15). Allergens have different structures and are classified in different protein families according to their biological function, suggesting that there may be few or no common structural features or intrinsic properties between allergens making them allergenic (18). However, it seems that there is an important connection between biological function, structural integrity and IgE binding capacity for an individual allergen to keep its allergenicity (15). Other factors cannot be ruled out as participants of the sensitization and the allergic reaction toward an allergen, such as genetic predisposition or defects in the regulation of IgE responses of the individual, other possible adjuvants such as hormones, bacterial and viral infections, and the route and degree of exposure (15, 49). In view of these implications, recombinant allergens altered by site-directed mutagenesis to remove IgE epitopes may represent a valuable tool for further research and more effective allergen immunotherapy.

Recombinant and Native House Dust Mite Allergens

Several characteristics describe the ideal recombinant allergen. An effective recombinant allergen must have IgE binding capacity comparable to its natural counterpart, ability to stimulate specific T cells and capacity to induce specific basophil degranulation. In addition, it must be capable of induction of IgE responses in experimental animals and induction of skin reactivity in humans (43). According to these desired characteristics, it has been shown that recombinant allergens offer several important advantages for the diagnostic and therapy of allergies. They can be produced in unlimited quantity and at reasonable costs, and the quality is not affected as has been shown in previous studies (52). These molecules can be more easily standardized, and the content of recombinant allergen cocktails could be accurately defined (46). Recombinant allergens also allow studying the spectrum of specificities of IgE, leading to the identification

of the specific allergenic components causing the allergic reaction in a patient. This means that the reactivity profile of the patient can be traced. In turn, this would allow tailoring the patient's therapy according to their allergen profile and will facilitate monitoring the sensitization to new allergens or the loss of reactivity toward distinct allergens. Another potential advantage of recombinant allergens is that they could be modified to produce derivatives or variants with reduced allergenic activity (18, 52). This means that the recombinant hypoallergenic derivatives would represent a lower risk of side effects or anaphylactic potential during immunotherapy. In addition, a long-term benefit from recombinant dust mite allergens might be the production of allergen variants that retain T-cell epitopes but no longer bind IgE (46). In this way, the possibility of side effects or anaphylactic reactions would be abolished. Many of the house dust mite allergens have been expressed as recombinant proteins and most of them have shown strong reactivity with IgE antibodies, equivalent to that found with native allergens (7, 18, 50). A study showed a strong correlation between RAST levels to native Der f 1 and Der f 2 and their recombinant counterparts (53). The recombinant allergens had almost the same IgE binding capacity as their native counterparts, although the native proteins had slightly higher RAST reactivity than the recombinants. The use of purified native and recombinant allergens has also allowed the identification of cross-reactivity among allergens of different species of house dust mites, a task that was not possible one or two decades ago (54). As established by the scientific literature, the use of recombinant allergens for studying the role of natural allergens in the allergic response as well as for diagnostic and immunotherapeutic purposes looks promising. Of course, it is essential to purify and analyze the natural allergens to compare their biologic activity with that of the recombinant ones and in this way establish a strong basis for the implementation of the recombinant allergens in research and therapy. Therefore, for a more effective and complete approach, research with house dust mites should be directed toward both, the characterization of native allergens, and the cloning and expression of their recombinant counterparts.

During the past decade, a variety of recombinant allergens have been produced through molecular biology techniques and the number of available recombinant allergens is increasing (43, 52). This achievement has allowed the identification and characterization of individual allergenic components of several house dust mite species. Molecular cloning has allowed identification of major allergens from the most common house dust mites such as Bt, Dp and Em. More than ten allergens have been cloned from the genus *Dermatophagoides* (family

Pyroglyphidae) and many have been expressed as recombinant proteins in bacterial or yeast cells (18, 55). Early in the 1990s, a group 1 allergen (Eur m 1) from Em was characterized resulting in approximately 85% sequence identity with Der p 1 (56). Many antigens from Df have also been characterized as important allergens, including Der f 1, Der f 2, Mag 1, Mag 3 and Mag 29. Interestingly, Mag 3 was found to have a high molecular weight of 177 kDa, opposing to the traditional findings that house dust mite allergens fall mostly in the range of 10-70 kDa (57). Many cross-reactivity studies have also been conducted with allergens from different species such as *Dermatophagoides* species, *Lepidoglyphus destructor* (family Glycyphagidae) and Bt (37, 38, 58, 59).

A recombinant protein from Bt designated Blo t 5 was cloned and expressed, finding approximately 43% homology with Der p 5 (molecular weight: 14 kDa) from Dp. The recombinant protein was screened with 139 individual sera from patients with asthma from Brazil, Florida and Puerto Rico, and it was found 69% with IgE antibodies to the recombinant protein, concluding that it was an important allergen. The cDNA clone codes for a mature protein of a predicted molecular mass of 13,876 Daltons, and it was the first Bt allergen to be cloned and fully sequenced. The investigators conclude that there is strong evidence to support that allergens from Bt are an independent causative agent of sensitization among patients with asthma living in tropical areas. Also, the study of IgE reactivity with Blo t 5 and Der p 5 supports the notion of Bt allergens as species-specific and therefore should be included in diagnostic procedures of dust mite allergies, particularly in tropical and subtropical regions of the world (60, 61). Later, in another study, Blo t 5 showed low to moderate levels of cross-reactivity (<60% cross-inhibition, with very high concentration of protein) with allergens from Dp, even with its counterpart, Der p 5, which shares 43% homology with Blo t 5. These results provided additional evidence for the independent role of Bt allergens as a source of sensitization (62).

In 1996, Caraballo and collaborators characterized at the molecular level an allergen designated Bt-M, using a cDNA library isolated from whole Bt. Through immunoadsorption and IgE binding frequency studies, they found that the recombinant protein shares at least one allergenic epitope with three native allergens of this mite, which have molecular weights of 11-13, 14 and 16 kDa and are considered important allergens. These investigators also found no cross-reactivity between Bt-M and group 2 allergens of Dp. However, they found that the predicted molecular weight of Bt-M is only 8,373 Daltons and the sequence for their clone has significant homology with Der p 5, which has a molecular weight of

13,585 Da, suggesting that they had identified a partial clone of Blo t 5 (63). Later, in 1998, Caraballo and collaborators found that Bt-M is the C-terminal segment of Blo t 5 and has significant cross-reactivity with Der p 5 (64). Puerta and collaborators analyzed a cDNA coding for another Bt allergen, called Bt11a, finding 50% frequency of IgE binding and concluding that it was an important allergen (65). Caraballo and coworkers reported the subcloning and sequencing of a cDNA clone from Bt called Bt6. This clone encoded a 130-amino acid protein of 14.8 kDa and showed 11% of IgE reactivity when tested with allergic patient sera (66). Further characterization of Bt6, which had been officially named Blo t 13, was performed. This allergen showed sequence similarity to a family of cytosolic lipid transport proteins (cLTPs) and therefore, represents the first mite allergen with a function in the fatty acid binding protein/lipocalin superfamily of allergens. That given that cLTPs have a highly conserved structure and a very important biological function, it is highly probable that other mite species have homologues to Blo t 13. The study of other mite species for such homologues could aid in understanding the biological function and allergenicity of this and other lipid binding proteins (67). Recently, two monoclonal antibodies against recombinant Blo t 13 were designed and their specificity for it was tested by immunoblotting. Both monoclonal antibodies recognized the same or close epitopes on the rBlo t 13 molecule. It is also found that *Dermatophagoides siboney* could have an allergen homologous to rBlo t 13 since it showed 84% inhibition of the binding of the monoclonal antibodies to rBlo t 13 in ELISA inhibition assays. These monoclonal antibodies facilitate further immunological and structural studies of rBlo t 13 (68).

Yi and coworkers reported the isolation and sequencing of a new allergen from Bt designated Blo t 10. This allergen is a tropomyosin with up to 96% of amino acid identity to other group 10 mite allergens such as Der p 10. Tropomyosin is a muscle protein that has been shown to be an important allergenic component in several invertebrate species. The investigators studied Blo t 10 simultaneously with Der p 10 and conclude that although they are highly conserved and show significant cross-reactivity among them, they do have unique IgE epitopes. Therefore, in regions where these two dust mites are prevalent, both of these allergens should be included in the diagnosis of dust mite allergy (69).

A group 1 recombinant allergen from Bt, designated Blo t 1, has been cloned and expressed (70). rBlo t 1 encodes a 221 amino acid mature protein with a molecular mass of 25 kDa. In addition, rBlo t 1 shares 35% identity with three house dust mite cysteine proteases previously characterized Der p 1, Der f 1 and Eur m 1. This Bt

recombinant protein exhibited 62% of IgE reactivity when tested with sera from Bt-positive patients. Therefore, rBlo t 1 is a major house dust mite allergen and it represents a useful tool for further research of the mechanisms involved in allergy and diagnosis of Bt hypersensitivity.

Recently, the cloning and molecular characterization of a cDNA from Bt homologous to dust mite group 3 allergens were reported (71). The clone, identified as Bt2-3, encodes a predicted mature protein of 231 amino acid residues with an estimated molecular weight of 27.5 kDa, and was shown to have significant sequence homology (48-54%) with other mite-derived serine proteases (trypsin-like proteases).

Another allergen from Bt is Blo t 11 which exhibits homology with different invertebrate paramyosins. rBlo t 11 reacted positively with 52% of sera from asthmatic patients exhibiting potentially important allergenic activity (72).

Conclusions

The allergenic role of Bt has been supported by several studies concerning the IgE binding capacity of Bt allergens and the cross-reactivity studies among Bt and other common house dust mites such as the Pyroglyphid members as well as other Glyciphagid mites. These studies have concluded that there is low cross-reactivity between allergens from these mites and that Bt possesses important species-specific allergens which have shown significant IgE binding frequency. It is important to include Bt in diagnostic and therapeutic strategies for house dust-mite induced allergy and asthma, particularly in areas, as Puerto Rico, where Bt exposure and sensitization are high. Therefore, there is a need for more studies regarding the allergens of this house dust mite and its role in allergies and asthma. This type of study, as well as other approaches toward the understanding of the allergic response to house dust mites, can be facilitated by the use of purified native and recombinant allergens. There is plenty of evidence presented by many researchers during the last two or three decades that recombinant DNA technology and other molecular biology and immunological techniques are very useful and promising tools in many areas of research and in medical applications.

Resumen

Los ácaros del polvo doméstico constituyen una importante fuente de alérgenos asociados con asma y condiciones alérgicas. El asma es una condición respiratoria que afecta a millones de individuos en todo el mundo y cuya prevalencia está en constante aumento. Las especies más comunes de ácaros domésticos incluyen

Dermatophagoides pteronyssinus (Dp), *Dermatophagoides farinae* (Df), *Euroglyphus maynei* (Em) y *Blomia tropicalis* (Bt). En las tres últimas décadas se han identificado y caracterizado a nivel molecular importantes alérgenos de estas especies; sin embargo, el estudio de los alérgenos de Bt ha sido menos intenso, a pesar de la importancia de este ácaro en regiones tropicales y subtropicales del mundo, incluyendo Puerto Rico. Es importante incluir a Bt en estrategias diagnósticas y terapéuticas, particularmente en áreas donde la exposición y sensibilización a Bt es alta. La tecnología de ADN recombinante, así como otras técnicas inmunológicas y de biología molecular, han jugado un rol fundamental en el estudio de la biología de los ácaros y su rol en el desarrollo de condiciones alérgicas y asma permitiendo conocer los mecanismos inmunológicos envueltos. La continuidad de investigaciones sobre alérgenos de ácaros domésticos permitirá el desarrollo de diagnósticos y terapias efectivas. El objetivo de esta revisión es describir los aspectos más importantes del rol de los ácaros como factor de riesgo de asma y alergia, y los últimos hallazgos sobre alérgenos de estos organismos, principalmente aquellos derivados de Bt.

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