

MEETING POINT**ANNUAL MEETING OF THE ARAGONESE SOCIETY
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This section publishes the works presented at the scientific meetings organised by Autonomic or Regional Allergy and Clinical Immunology Societies. In this issue the Section contains the communications to the first two main subjects of the Annual Meeting of the Aragonese Society of Allergology, held in Formigal in February 1999. Papers corresponding to this Section which have been published previously in other Sections of this Journal, as well as those published in other journals, appear only as references.

**FIRST MAIN SUBJECT:
BIODIVERSITY OF DUST
MITES IN ALLERGOLOGIC
PATHOLOGY**

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Mites and their allergens

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INTRODUCTION

In the industrialised countries, most individuals spend over 95% of their time within closed environments, where the air may contain pollutants and contaminants at higher concentrations than those found in the open air. For this reason, the quality of the air in closed environments is presently considered to be at least as important as that of the open air for health in general and for allergic asthma, rhinitis and conjunctivitis in particular.

House dust is the main source of allergens within the households. This complex material comprises inorganic matter and organic matter inclu-

ding fibres, mould spores, bacteria, pollens, insects, mammal epithelia and mites. The progressive increase currently observed in the frequency of atopic diseases¹ might be linked to a greater exposure to allergens within the households, and also to the materials used in the buildings and to their type of construction. Buildings and dwellings are at the present time constructed with the aim of a greater efficiency in energy conservation, and this leads to a reduction of ventilation and airing and to a greater accumulation of allergens in the enclosed environment.

Genetic predisposition is also a fundamental conditionant of the susceptibility to suffer allergic respiratory diseases, but such diseases would never become manifest in the absence of environmental factors. Among these environmental factors, exposure to environmental allergens is of prime importance^{2,3}. Exposure to high levels of allergens during the early months of life increases the risk of sensitization and of developing allergic asthma^{4,5}. Contrary to this, environmental control measures implemented shortly after birth reduce the frequency of allergic symptoms in childhood².

Specific immunoassays are currently available for the quantification of allergens in the environment, and it is possible to measure the concentrations of mite, cockroach, mould and cat allergens in the house dust. The data derived from such measurements have provided highly important information regarding the presence and distribution of environmental allergens in the house dust and in suspension in the air, and also regarding the effectiveness of the environmental control measures.

BIOLOGIC ACTIVITY OF ALLERGENS

An allergen is an antigen that is able to induce an IgE-mediated response. Allergens are generally proteins or glycoproteins with molecular weights ranging between 5 and 100 kDa. The sources of allergens are quite varied, and include mammals, arthropods, pollens, moulds, bacteria and a host of other possibilities. A single such allergen source contains many proteins with varying allergenic capacities. Once the allergen has come into contact with the immune system, it induces a response mediated by T- and B-lymphocytes with antibody production. In an allergic patient, these antibodies are mainly of the IgE isotype. This response is regulated by the T-lymphocytes, and both the antibodies and the T-lymphocytes participate in the development of the allergic disease, B epitopes being recognised by the antibodies and T epitopes by the T-lymphocytes in the context of Class II molecules of the Main Histocompatibility Complex (HLA, DR, DP, DQ).

The *in vitro* effects of the mite allergens have been studied by a number of authors. Release of mediators was first demonstrated in adults with atopic dermatitis and mite hypersensitivity. Der p1 concentrations of 6 to 10 mg/ml induced histamine release from the peripheral blood basophils in such individuals⁶. A study in asthmatic children disclosed a similar pattern, although the peak histamine release occurred with Der p 1 concentrations in the range of 2 to 10 mg/ml⁷. A release of chemotactic factors such as the platelet activation factor (PAF) has also been demonstrated in basophils from asthmatic subjects with mite hypersensitivity stimulated with mite extracts. In such individuals, the application to the skin of mite allergens induces migration of basophils and eosinophils to the site of application⁶. It is also known that bronchial challenging with allergens increases the expression of adhesion molecules (ICAM-1) in the bronchial epithelium, together with increases of the numbers of circulating eosinophils, basophils and stem cells⁸ and of the number of eosinophils in the bronchoalveolar lavage fluid⁹. A number of studies have demonstrated that mite allergens are able to induce t-lymphocyte proliferation *in vitro*. Clones of mite-specific T-lymphocytes

have been isolated that respond to such allergens^{10,11}. Mite allergen-specific clones of T-lymphocytes from atopic individuals induce IgE synthesis *in vitro*. Those clones have the Th-2 phenotype and produce IL-2, IL-4 and IL-5 but not γ -IFN when stimulated with mite allergens. This fact might explain the origin of the predominantly IgE-type response in individuals with a genetic predisposition to allergic respiratory diseases.

The inhalation of mite allergens causes bronchoconstriction and wheezing in sensitized adults and children. This response is dose-dependent, and may be early or late^{12,13}. In the particular case of mites, the faecal particles contain *circa* 0.1 ng Der p 1. The allergen elutes rapidly on the surface of the mucosa and may achieve local concentrations of several mg/ml, which may be much higher than that required for stimulating histamine release from the basophils. If sufficient faecal particles are inhaled, the cumulative effect may produce airway obstruction. Inhalation of such high concentrations of the allergen may be important for the development of an inflammatory response and for the subsequent increase of bronchial reactivity.

The environmental exposure to *D. pteronyssinus* and its correlation to the presence of Der p 1 in the bronchoalveolar lavage fluid has been investigated in asthmatics who are allergic to the mite¹⁴. After overnight exposure to concentrations between 13.4 and 27.3 μ g Der p 1 per gram of dust in rugs and beds, respectively, a mean concentration of 3.4 ng/ml Der p 1 was detected in the bronchoalveolar lavage fluid of the individuals exposed to these environmental concentrations. In the same study, endobronchial challenge with 5 – 60 ng Der p 1 induced eosinophilia in the airways. These results clearly demonstrate that considerable amounts of Der p 1 can be detected in the bronchoalveolar lavage fluid when there is high exposure to *D. pteronyssinus* allergens in the bedroom. It is now known that most of the allergens from mites are proteolytic enzymes related to their digestive processes. It is also suspected that this enzymatic activity, which might behave as an adjuvant, might be involved in the high rates of sensitization to mite allergens which are frequently observed in countries with

mean relative humidity levels in excess of 50%. It has been recently demonstrated that Der p 1 may separate the cellular CD₂₃ and CD₂₅ receptors.

ALLERGEN NOMENCLATURE

The allergenic extracts currently in use in clinical practice contain all or most of the allergens present in the source of origin. Several allergens have been characterised and purified (Table I) and are currently used in the study of allergic response regularisation and in the quantification of environmental allergens. Those allergens inducing a specific IgE response in over 50% of the allergic population are termed "major allergens".

The World Health Organisation has established a systematic nomenclature system for the denomination of allergens¹⁵. The purified allergens are thus designated by the first three letters of their genus, the first letter of their species and an Arabic number assigned according to the chronological order of identification. The same number is usually used for designating allergens that are homologous or correspond to a related species.

MOLECULAR BIOLOGY

In their application to the study of allergens, the techniques of molecular biology have allowed us to obtain monoclonal antibodies and highly purified recombinant allergens through gene cloning, and also to identify similarities between the structures and the biologic activities of various allergens. A number of products obtained through these technologies are used in the standardisation of allergenic extracts, in the study of B and T epitopes and in the improvement of the methods for environmental allergen quantification. All these achievements have led to a better understanding of the pathogenesis of allergic diseases and to proposing new strategies for their study and treatment.

In cloning an allergen, a defined general strategy is followed that begins with the isolation of the messenger ribonucleic acid (mRNA) from

Table I. Allergens from domestic mites

Source	Allergens	Molecular weight (kDa)
Group 1	Der p 1, Der f 1, Eur m 1, Der m 1	25
Group 2	Der p 2, Der f 2, Eur m 2, Tyr p 2	14
Group 3	Der p 3, Der f 3, Eur m 3	28-30
Group 4	Der p 4	56-63
Group 5	Der p 5, Blo t 5	14
Group 6	Der p 6	25
Group 7	Der p 7, Der f 7	22
Group 8	Der p 8	25
Group 9	Der p 9	24-28
Group 10	Der p 1, Der f 10	37
Group 11	Der f 11	98
Group 12	Blo t 12	14
Group 13	Blo t 13	14,8

*All these allergens have recognition rates ranging between 10% and 90% of the patients with mite allergy.

the natural source. From this mRNA, a complementary deoxyribonucleic acid (cDNA) chain is prepared through reverse transcription, and from this cDNA chain a double-stranded DNA is synthesised through a DNA polymerase reaction. The synthesised DNA is introduced into a vector, which may be a plasmid or a bacteriophage, with the help of restriction enzymes. An *E. coli* strain is then infected or transformed with the recombinant vector. The set of clones containing the cDNA derived from the allergen source is termed a cDNA or genomic library. Once such a library has been established, the clones containing cDNAs codifying for an allergen of interest are selected. This selection is performed with the help of antibodies that react with the recombinant protein expressed by the cloned gene, using sera from allergic patients with high titres of specific IgE for the cloned allergen, or using monoclonal antibodies. Another method of clone selection is that of hybridisation, in which labelled oligonucleotides (probes) are used which recognise and adhere to the complementary nucleotide sequence in the cloned gene. Once the clone containing the cDNA of interest has been selected, it can be used for a number of purposes: (a) for producing large quantities of highly purified allergen, or (b) for the determination of

the nucleotide sequence codifying for that allergen. From the nucleotide sequence, the aminoacid sequence of the cloned allergenic protein is then inferred. The deduced aminoacid sequence is then analysed in order to determine if it shows homology to that of other proteins that may be found in databases such as that of the U.S. National Institutes of Health (Genbank) or that of the European Molecular Biology Laboratory (EMBL).

The availability of the cDNA facilitates the study of those regions in the molecule which are particularly important for the biologic activity. For the determination of antibody binding sites, or of sites of binding to the T lymphocyte receptor, a technique known as "epitope screening" is used. In this method, the gene codifying for the allergen is fragmented either with the help of restriction enzymes or through physicochemical methods. The resulting fragments are cloned in an expression vector, and the allergenicity of the resulting products is assessed through immunoassay procedures. Using this methodology, Der p 1 has been demonstrated to have five B epitopes, of which three are shared by Der f 1¹⁶.

MITE ALLERGENS

Household mites represent the main source of allergens in indoor house dust and are also among the commonest sensitizing agents in childhood. The allergenic activity in house dust was discovered in 1965 to be due to mites of the *Pyroglyphidae* family, *Dermatophagoides pteronyssinus* and *D. farinae*¹⁷. More recently, the allergenic role of other species such as *Euroglyphus maynei*, *Blomia tropicalis*, *Lepidoglyphus destructor*, *Glycyphagus domesticus*, *Aleuroglyphus ovatus*, *Tyrophagus putrescentiae*, *Acarus siro* and *Chortoglyphus arcuatus* was demonstrated. These mites are currently termed "household mites". Important studies have also been carried out in emergency wards showing that exposure to mite, cockroach, *Alternaria* and cat allergens are outstanding risk factors for asthma exacerbations¹⁸⁻²¹. The faecal particles shed by mites carry most of their allergens. These particles are spherical in shape, with a diameter ranging between 10 and 40 μm , and are easily suspended and borne in the air²². The

allergens are processed in the mites' gut. Guanine detection represents an alternative method for estimating the quantity of mite allergen in house dust, as this purine base is the main nitrogenated product in the faeces of these arthropods²³.

D. pteronyssinus has over twenty allergens, which are recognised with varying frequency and intensity by the sera of atopic patients²⁴. A number of these allergens have been characterised and grouped according to their physicochemical similarities.

Group 1:

The Group 1 allergens of *Dermatophagoides*, Der p 1, Der m 1 and Der f 1, have a 24 kDa molecular weight and 80% chemical homology among themselves²⁵. The Eur m 1 allergen of *E. maynei* also belongs to this group. These allergens show a 80 – 90% frequency of recognition by allergic patients. Der p 1 was initially cloned in *E. coli*, and the sequence of the gene revealed that this allergen has homology to actinidine, papain, cathepsin H and cathepsin B, a group of proteases with a cysteine residue in the active moiety²⁶. Because of the weak antigenic capacity of the allergen expressed in *E. coli*, the Der p 1 gene was expressed in *Saccharomyces cerevisiae*, evidencing greater reactivity against sera of mite-allergic patients²⁷. The group 1 allergens are cysteine proteases that are able to separate the low affinity IgE receptor (CD₂₃) from the surface of human B lymphocytes²⁸. This receptor is also present in eosinophils, follicular dendritic cells, macrophages and platelets. Because the soluble CD₂₃ promotes IgE synthesis, it is assumed that the CD₂₃ fragments released by the action of Der p 1 might potentiate the synthesis of this immunoglobulin. Der p 1 has also been demonstrated to separate the μ subunit of the interleukin 2 receptor (IL-2R, or CD₂₅) from the surface of human peripheral T cells; as a consequence, these cells evidence less proliferation and less γ -interferon secretion in response to anti-CD3 stimulation²⁹. The authors of these studies conclude that as IL-2R is very important for the prolongation of Th1 cells, its separation by Der p 1 might induce a Th2 immune response. The separation of CD₂₃ and CD₂₅ by the action of Der p 1 potentiates its allergenicity, establishing a microenvironment with a clear trend towards a Th2 response³⁰.

Group 2:

The group 2 allergens are 14-kDa proteins; about 80% of all patients allergic to mites have specific IgE against these allergens³¹. The gene expressing Der f 2 codifies for a 129-aminoacid protein with a molecular weight of 14 kDa. This sequence has 88% homology to that of Der p 2³². The gene expressing Der p 2 codifies for a 122-aminoacid, 14.1-kDa protein. Der p 2 has been efficiently expressed as a fusion protein in *E. coli*, and the recombinant protein retains most of the allergenic activity evidenced by the native one^{33,34}. Studies with recombinant Der p 2 peptides have shown that the Der p 2 antigenic determinants are conformational ones, and that fragments of less than 95 aminoacids do not express allergenic activity³⁵. Group 2 allergens have been reported to have *ca.* 30% homology to a family of epididymal proteins which includes the human, bovine and chimpanzee ones³⁶. The allergens Tyr p 2 from *T. putrescentiae*, Lep d 2 from *L. Destructor* and Eur m 2 from *E. maynei* also belong to this group.

Group 3:

Der p 3 and Der f 3 are proteins with molecular weights between 24.9 and 30 kDa with activities of the serine protease (trypsin) type and 50% homology to other serine proteases. The complete gene codifies for a 261-aminoacid protein, indicating that Der p 3 is synthesised as a pre-prozymogen, similarly to other trypsines. Group 3 includes several isoforms, and *ca.* 60 to 80% of the patients allergic to *Dermatophagoides* spp evidence IgE against these proteins³⁷. Eur m 3 also belongs to this group.

Group 4:

Der p 4 and Der f 4 form group 4 of mite allergens. The molecular weights of these allergens range between 56 and 63 kDa, and they have a frequency of IgE binding of between 24 and 46% in children and adults, respectively. These allergens have homology to amylase³⁸.

Group 5:

Der p 5 is a 14-15 kDa allergen with a frequency of binding to specific IgE below 20%.

This allergen has been cloned and has evidenced allergenic activity in skin tests in 60% of the asthmatic individuals studied^{39,40}. One allergen from *Blomia tropicalis*, Blo t 5, has 43% homology to Der p 5 and a 45 to 60% recognition frequency⁴¹.

Group 6:

Der p 6 and Der f 6 constitute group 6. These allergens have a molecular size of 25 kDa and serine protease chymotrypsin enzymatic activity⁴². Their IgE recognition frequency ranges between 40 and 60%. Der p 6 has been shown to have 37% homology to Der p 3.

Group 7:

Der p 7 and Der f 7 share 86% homology and evidence a high degree of cross reactivity. Der p 7 is a 198-aminoacid protein with a molecular weight of 22.1 kDa. Der p 7 has also been cloned, and the recombinant allergen has demonstrated allergenic activity in skin tests and RAST, with positive results in 53% and 37%, respectively, of the allergic patients studied⁴³. Antibodies against this allergen also react in Western blots with multiple bands with molecular weights of 29, 27 and 24 kDa; these results have been confirmed in absorption experiments. Six isoforms of Der p 7 have been identified.

Group 8:

The molecular weight of the Der p 8 allergen is 25-26 kDa, and it has a *ca.* 40% recognition frequency⁴⁴. This allergen shows considerable homology to the rat and mouse glutathione-S-transferase, and 25% homology to Bla g 5, an important allergen from *Blatella germanica*. There is apparently no cross reactivity between both allergens.

Group 9:

Der p 9 has a molecular size of 24 to 29 kDa. It has three isoforms (pI 4.8, 7.8 and 10.5). It is recognised by *ca.* 80% of the patients with *D. pteronyssinus* allergy, and has collagenolytic activity⁴⁵. Der p 9 has slight cross reactivity with Der p 3 and Der p 6 in RAST inhibition experiments.

Group 10:

Der p 10 and Der f 10 correspond to the acarine tropomyosin, and their molecular weight is 33 to 37 kDa. They evidence 76.1%, 58.8% and 58.1% homology to the *Drosophila melanogaster*, rabbit and human α -tropomyosins, respectively, and a rather high recognition rate by IgE (>60%)^{46,47}. These allergens appear to be involved in the cross reactivity process between mites, shrimp and insects in patients with shrimp allergy.

Group 11:

This group is comprised of allergens with sequence homology to paramyosin, a structural protein of muscle in invertebrates. Der f 11 has a 98-kDa molecular weight and a high rate and intensity of IgE recognition⁴⁸.

Groups 12 and 13:

These two groups were instituted in order to include purified *B. tropicalis* allergens. Blo t 12 has a 14-kDa molecular weight and 50% IgE recognition rate. Blo t 13 has a 14.8 kDa molecular weight and 10% IgE recognition rate. This last allergen shows considerable sequence homology to cytosolic fatty acid-binding proteins^{49,50}.

CROSS REACTIVITY BETWEEN MITE ALLERGENS

Many individuals with mite allergy evidence sensitization to multiple species, and this is in part due to the cross reactivity of allergenic determinants shared between the species

involved in the allergic response⁵¹. A high degree of cross reactivity exists between *D. pteronyssinus* and *D. farinae*, as reflected by the fact that individuals with *Dermatophagoides* allergy evidence skin sensitization to both species. On the other hand, cross reactivity between *Pyroglyphids* and non-*Pyroglyphids* is minimal or absent^{20,52-54}. *B. tropicalis* evidences minimal cross reactivity with *Dermatophagoides* and high cross reactivity with *L. destructor*^{20,54,55}. Nasal challenge and immunoblot studies using *B. tropicalis* extracts demonstrate that this allergen is able to induce a specific allergic response⁵⁶. This species plays an important role as inductor of respiratory allergy in regions where its presence is frequent. In regions where *B. tropicalis* is abundant and *L. Destructor* does not exist, sensitization to the latter species is quite probably due to allergenic *B. tropicalis* epitopes with cross reactivity to *L. destructor*. The use of *B. tropicalis* extracts is a requisite for diagnosis and therapy in regions where this species is abundant.

ENVIRONMENTAL CONTROL OF MITES AND OF THEIR ALLERGENS

The measures for the environmental control of mites and of their allergens may be divided into two main headings:

(1) Methods for killing the mites (use of acaricides and environment modification measures which directly affect their survival, such as humidity reduction, temperature increase and freezing).

Table II. Ideal environmental control measures

1. Eliminate old pillows, rugs, drapes, curtains, toys and books from the bedroom of the allergic patient.
2. Eliminate rugs from the whole household (and do not use them).
3. Clean first with a powerful vacuum cleaner, and then cover the mattress, pillows and blankets with anti-mite plastic covers.
4. Wash the pillows, mattress covers and bed linen once a week at temperatures >50°C. ideally, a liquid acaricide should also be used.
5. Use acaricides when the rugs cannot be removed. Acaricides can also be used for preventing mite colonisation.
6. Vacuum-clean sofas, settees and other upholstered furniture at weekly intervals.
7. Use insecticides to combat cockroaches and improve hygiene.
8. Reduce the absolute humidity level to below 7 g/kg or the relative humidity level to below 50% with the help of dehumidifiers or air conditioning. The use of dehumidifiers and air conditioning reduces the mite populations.
9. Clean the air conditioning systems at regular intervals.

(2) Methods for the isolation, elimination, denaturation or inactivation of the allergens (use of vacuum cleaners, wet cleaning, chemical denaturation with tannic acid and use of plastic covers).

The main environmental control measures are shown in Table II. Control of the allergens in a household is not achieved by using one single such measure: a set of measures aimed to the complete environment within the household is required for the control of exposure to mites and to their allergens.

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Clinical impact of the mite species in atopic patients

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INTRODUCTION

Studies performed in various parts of the world have demonstrated a high prevalence of mites in the indoor house dust^{1,2}. In many places, the dust mites predominate in so-called house dust allergy and appear as the responsi-

ble agents in up to 85% of all cases of asthma³⁻⁶.

Sensitization to mites is directly related to the amount of major *Pyroglyphid* mite allergens in the dust samples, as is also the severity of the symptoms of allergy. It has been demonstrated that the mite levels within the house may change in relation to the seasons or to changes in the house furniture. The mite density is quite variable between the different places sampled within a household, regardless of the sampled homes being within the same geographical area. This within-house variability probably reflects the varying availability of food for the mites (mainly skin scales).

Fernández Caldas has reported, in his work in Cartagena (Colombia)^{7,8}, that Der p 1 levels in excess of 1.14 µg/g are associated to a 95% or greater probability of eliciting positive skin tests or RAST to *Dermatophagoides pteronyssinus*. It is possible that in areas where several different acarine species coexist the Der p 1 levels required for inducing sensitization may be lower.

Besides the *Pyroglyphid* mites, a large number of different allergenically significant mites has been documented, collectively known as "storage mites". These mites may be found in areas where grain is stored, in straw and in grain silos. Among these storage mites, the predominant families are the *Glycyphagidae* and *Acaridae* ones, including the genera and species *Lepidoglyphus destructor*, *Tyrophagus* and *Acarus siro*. Storage mites have been demonstrated to be the cause of bronchial asthma, allergic rhinitis and allergic conjunctivitis, particularly in rural environments with occupational exposure, such as in farms, dairy farms, granaries, bakeries and silos with grain elevators.

Considerable attention has been dedicated in recent years to storage mites in non-occupational environments, and the results point out that sensitization to these agents is not restricted to the cases with occupational exposure^{9,10}.

The lowest relative humidity level at which mites are still able to maintain their water balance and survive is influenced by the ambient temperature and ranges between 55%