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REVIEW

Allergic and nonallergic interactions between house dust mite allergens and airway mucosa

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Allergic and nonallergic interactions between house dust mite allergens and airway mucosa. N. Roche, T.C. Chinet. G.J. Huchon. ©ERS Journals Ltd 1997.

ABSTRACT: Asthma and allergy are extremely frequent diseases, affecting 5–10% and 30% of the population, respectively. The prevalence of asthma has increased in many developed countries, which may be due to several factors, including increased exposure to house dust mite (HDM) allergens.

HDM to which humans are most frequently sensitized are *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and *Euroglyphus maynei*. These mites multiply in carpets, bedding and upholstered furniture in a hot and humid atmosphere. The allergens are digestive enzymes of the mites. Several epidemiological studies have shown that an increase in exposure to HDMs is associated with an increase in the prevalence of sensitization and asthma, whereas mite avoidance leads to a decrease in respiratory symptoms of sensitized asthmatic subjects.

Sensitized subjects have specific immunoglobulin G and E (IgG and IgE) humoral responses, as well as proliferative T-cell responses to HDM allergens. Experimental exposure to HDM allergens induces bronchoalveolar inflammatory responses, that are characterized by the recruitment and activation of eosinophils, mastocytes, neutrophils, monocytes and lymphocytes. The cysteine protease activity of *Der p* 1 (a major allergen of *D. pteronyssinus*) has been shown to increase airway mucosal permeability, and may thereby contribute to the pathogenesis of airway inflammation and hyperresponsiveness by nonimmunological mechanisms.

These epidemiological and experimental data support the recommendations for mite avoidance, especially in persons at high risk of developing asthma.

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Asthma is a chronic bronchial disease, which is characterized by airway inflammation and bronchial hyperresponsiveness (BHR) to various stimuli, leading to paroxysmal symptoms, such as cough, wheezing and dyspnoea [1]. In a given subject, the occurrence of asthma depends both on genetic predisposition and exposure to environmental factors [2]. More than 90% of asthma cases are associated with an atopic status. The prevalence of asthma in epidemiological studies varies from 5 to 25%, and is increased in many populations [2]. Many factors may explain this increasing prevalence, such as: better recognition of the disease; increase in the number of genetically predisposed subjects; or changes in environmental factors, such as pollution, diet, or allergen exposure. Sixty to 100% of asthmatics are sensitized to house dust mites (HDMs), and the implication of these allergens in sensitization, bronchial hyperresponsiveness and asthma has been extensively studied.

Many authors have recently hypothesized that these allergens are not only capable of provoking asthma attacks in sensitized patients, but may also be causally linked to the occurrence of asthma in some individuals. According to this hypothesis, the association between exposure to HDM allergens and asthma follows a two step process: firstly, early exposure leads to sensitization; and, secondly, repeated exposure leads to airway inflammation, BHR and symptoms. To determine whether such a causal association is likely, several questions have to be answered [3–5]. The first series of questions can be addressed by epidemiological studies: 1) Is there an association between exposure to HDM allergens and the occurrence, persistence and progression of asthma? 2) Is this association strong, consistent and independent of other risk factors? 3) Does exposure precede the onset of asthma? 4) is there a dose-dependent association between the level of exposure and the severity of asthma? Clinical experimental studies may make it possible to answer a second series of questions: 1) Is there an experimental relationship between exposure and symptoms? 2) Is there an experimental relationship between sensitization and symptoms? Finally, experimental laboratory studies will address the last question: Are these plausible biological mechanisms of asthma induction by HDM allergens?

After describing HDMs and their allergens, we will review epidemiological and clinical evidence for the association between HDM allergens and asthma, and laboratory evidence showing that HDM allergens are capable of inducing not only airway inflammation but also direct epithelial injury. Despite these increasing data, several questions remain unanswered, especially concerning the respective contributions of exposure to HDM allergens

and other environmental and genetic factors to the pathogenesis of asthma, and the interactions between these factors.

House dust mites

Classification and way of life [6–8]

Comman house dust mites belong to the family Polyglyphidae of the order Acarina, which itself belongs to the class Arachnidae. The main family of mites associated with human atopic diseases is Pyroglyphidae, which contains 47 species in 17 genera. Thirteen species of Pyroglyphidae are contained in house dust, where they cohabit with storage mites of other families, *i.e.* Acaridae and Glycyphagidae. In most regions of the world, the predominating species of Pyroglyphidae are *Dermatophagoides pteronyssinus*, *D. farinae* and *D. microceras*, and *Euroglyphus maynei*. In Europe, *D. pteronyssinus* (*Der p*) is the most frequently encountered HDM. Allergens from these mites are secreted in their faeces [9].

Mite growth is greatly influenced by relative humidity and temperature. Food sources of mites include skin scales or fungi that grow on them. Growth of Pyroglyphidae is limited by predation by other mites, and excessive fungal growth inhibits growth of cultured mites; however, these factors are unlikely to be relevant in the home. HDMs are ubiquitous; they are found primarily in bedding, upholstered furniture, and carpets. Many recent changes in the human environment are likely to be responsible for an increase in the "mite load" of our homes: vacuum cleaners have replaced picking up and beating carpets; increased use of indoor heating and air humidification systems provides optimal environmental conditions for mite growth (*i.e.* temperature at 17–25°C and relative humidity >50%), especially when ventilation is reduced to save energy; and new detergents that have no acaricide properties are used to wash bedding in cool water, which does not affect mites. However, other factors tend to decrease mite growth, such as central heating, which induces drying of indoor atmosphere when not associated with humidification systems.

Allergens and their measurement and standardization

The most studied species of HDMs are *D. pteronyssinus* and *D. farinae*. Allergens of these mites have been purified, identified and characterized by biochemical and immunological techniques, including: isoelectric focusing; gel filtration; cross-radioimmunoelectrophoresis; immunoblotting; and affinity chromatography, using monoclonal antibodies [6, 10–12]. The complementary deoxyribonucleic acid (cDNA) of some of these allergens has subsequently been cloned [13, 14]. Two major groups (1 and 2) of Dermatophagoides allergens have been identified; they are considered to play a major role in human disease because most mite-allergic patients (*i.e.* 70–100%) demonstrate immediate hypersensitivity to them. Their molecular weights are 25 and 14 kDa, respectively, and their isoelectric points are heterogeneous and overlapping. Seven other groups [3–9] have been identified, but seem to be less frequently involved in human sensitization. Within each group, allergens have common physicochemical characteristics (*e.g.* molecular weight) and amino acid sequences, making them structural homologues. Most of them are actually digestive enzymes of the mites, which are secreted in their faeces [13, 15–21] (table 1). These allergens also share cross-reacting epitopes for antibodies of hyperimmunized animals (polyclonal antibodies from rabbits and murine monoclonal antibodies) and of mite-sensitive atopic patients.

Table 1. – Biochemical properties of the allergens of *Dermatophagoides pteronyssinus* (*Der p*) [22, 23]

Allergen	MW kDa	Enzymatic activity
Der p 1	25	Cysteine and serine protease
Der p 2	14	Lysozyme-like?
Der p 3	25	Trypsin
Der p 4	56	Amylase
Der p 5	17	Not identified
Der p 6	30	Chymotrypsin
Der p 7	22	Not identified
Der p 8	28	Serine protease

MW: molecular weight.

Measurement of allergen concentrations in dust samples, mite cultures or commercial allergen extracts can be performed either by nonspecific methods, *i.e.* radioallergosorbent test (RAST) inhibition that measures total allergen content, mite counts or measurement of guanine concentration [6], or by allergen-specific techniques such as enzyme-linked immunosorbent assay (ELISA), immunodiffusion, inhibition radioimmunoassay (RIA), immunoelectrophoresis or monoclonal assays. Allergen-specific techniques using monoclonal antibodies are now widely used because they are sensitive, specific, reproducible, and can be automated for use in large scale studies. Measurement of guanine concentration is simple and inexpensive, but not species-specific, and less reliable than immunochemical assays; mite count is time-consuming and requires trained technicians. Standardization of allergen extracts relies on the World Health Organization (WHO) international standard (IS) reference (called NIBSC 83/518) that was established at the end of the 1980s, following several international collaborative studies using skin testing, RAST inhibition and monoclonal assays [6].

Epidemiological links between HDM and asthma

Several cross-sectional and longitudinal observation studies have established a dose-dependent relationship between: 1) the levels of HDM allergens in the environment; and 2) the prevalence of atopy, sensitization to these allergens, bronchial hyperresponsiveness and asthma [24]. However other studies have not confirmed these results [25]. In some serial cross-sectional studies, the same population has been studied at two different periods of time, which were separated by modifications in the environment that led to an increase in the level of exposure. These studies have the major advantage of reducing an important potential source of bias, *i.e.* differences in genetic predisposition. However, such a bias cannot be eliminated, nor can the role of other environmental factors, such as air pollution, dietary intake, or exposure to other allergens [2].

Relative risk of sensitization and HDM levels

The relative risk of sensitization to HDM, assessed by skin-prick tests and/or specific serum immunoglobulin E (IgE), increases when levels of HDMs increase. PLATTS-MILLS and co-workers [7] found that the increase in the risk of sensitization becomes significant at a threshold of 2 μg of group 1 HDM allergens (which corresponds approximately to 100 mites)·g^{-1} of dust. This finding has been confirmed by several other studies in various geographical areas [26–28]. In a study by LAU *et al.* [26], the risk was 32-fold higher in highly exposed atopic subjects (>10 μg of group 1 allergen·g^{-1} of dust) than in the lowest exposed atopic group (<0.4 μg·g^{-1} of dust); the threshold of 2 μg·g^{-1} of dust was associated with a five-fold increase in the risk of sensitization.

However, levels of exposure were similar in atopic and nonatopic groups, suggesting that allergen exposure alone cannot induce atopy, or that much higher concentrations of allergen are necessary. This has been confirmed by a large prospective study of 1,802 children, which showed that the increase in sensitization becomes significant at 2 μg of group 1 allergen·g^{-1} of dust in previously atopic children, whereas a much greater concentration of 80 μg·g^{-1} was required in children who had no previous positive skin-prick test [29].

In Sweden, where HDM infestation used to be uncommon, HDM sensitization seems to be increasing in parallel with exposure. HDM content of mattresses is higher in HDM-sensitized children, in whom asthma is more frequent, than in non-HDM-sensitized atopic control children [30]. Higher HDM contents are associated with more recent insulation installations. Interestingly, HDM sensitization has been observed in some children who were exposed to lower levels of mites than the threshold of 2 μg of group 1 allergen·g^{-1} of dust, indicating that this value should only be considered as a statistical threshold and cannot be applied to individual subjects. Regarding the potentially confounding role of genetic predisposition, a study of 21 sibling pairs is of particular interest [31]; this study showed that HDM-sensitive children were exposed to higher levels of HDM than their non-HDM-sensitive siblings.

Relative risk of asthma and HDM levels

The initial data come from cross-sectional population and case-control studies. In many countries, sensitization to HDM is present in the majority of asthmatics, in whom it is far more common (94%) than in the general population (32%) [32]. In children, at least such sensitization has been associated with an impairment of lung function [33]. In the south of France, skin sensitization to HDM allergens and asthma and related symptoms have been found to be significantly more prevalent in a population living at sea level (prevalences 27.5 and 4.1%, respectively) than in another population living at 1,500 m of altitude (prevalences 10.2 and 2.4%, respectively), where the HDM content in mattress dust samples is lower [34, 35]. A study of schoolchildren in these two regions found that the global prevalence of asthma and allergic rhinitis did not differ between the two regions [36], but the prevalence of asthma together with a positive skin-prick test to HDM was higher in children living at sea level than in children living in and native to the mountain region (this difference was not found between children living at sea level and children living in but not native to the high altitude region, which suggests differences in genetic predisposition or an influence of early exposure to HDM). In this study, the absence of difference in the global prevalence of asthma is likely to be related to a higher prevalence of sensitization to pollens in the mountain region [36]. In mite-allergic asthmatics from the sea level town, a high amount of HDM in samples of mattress dust was associated with poor asthma control and a higher beta$_2$-agonist requirement [37].

Such influences of environment on HDM exposure and on the subsequent development of asthma were found in several other studies, which demonstrated that the relative risk of asthma was related to the level of exposure to HDM allergens in a dose-dependent fashion [4, 27, 28, 38–40]. One of these studies found that the risk of asthma in HDM-sensitized children doubled with every doubling of *Der p* 1 levels [4]. Differences in home HDM content between asthmatics and controls can be explained by climatic differences in some cases, and by differences in indoor environment in others (such as the introduction of good conditions for mite growth, *e.g.* woollen blankets) [38]. In these studies, when compared to sensitization to various other allergens, sensitization to HDM allergens was the strongest determinant of the onset of asthma [28, 40].

Australian authors also performed large serial cross-sectional studies based on questionnaires and measurements of domestic dust HDM content, bronchial hyperresponsiveness and skin sensitivity, to allergens. These studies showed that an approximately five-fold increase in HDM levels was associated with a 1.5–3 fold increase in wheezing and a similar increase in documented BHR, whereas the prevalence of atopy remained stable [41]. The increase in BHR was observed mainly in atopic subjects.

Finally, prospective studies have demonstrated a relationship between the level of exposure to HDM during childhood and the presence of asthma in childhood and adulthood, the age of onset of asthma being lower when HDM levels were higher [32]. Such studies have made it possible to assess the temporal relationship between exposure (especially during infancy) and subsequent occurrence of the disease [32, 41]. Other prospective studies have found that seasonal variations in HDM exposure were related to variations in BHR in asthmatic patients, with maximum levels of HDM and BHR being measured during late summer and autumn [42].

Taken together, the data from these epidemiological studies have led to the consideration of two levels of risk: exposure to 2 μg of group 1 HDM allergens·g^{-1} of dust is associated with an increased risk of sensitization and BHR, whereas, exposure to 10 μg·g^{-1} of dust is associated with an increased risk of exacerbations of asthma. Again, it must be emphasized that these are statistical thresholds, which have only a limited significance in a given individual, in whom personal exposure to HDM allergens is combined with other risk factors for asthma development.

Clinical experimental links between exposure and sensitization to HDM and asthma

Whilst epidemiological studies support a role for HDM allergens in the genesis and subsequent severity of asthma, clinical experiments make it possible to study two aspects of the effects of HDM in asthma: firstly, the relationship between exposure and clinical response in patients who already suffer from asthma, *i.e.* the role of these allergens in BHR and symptoms, and the effect of avoidance measures on asthma severity and control; and, secondly, the role of sensitization to these allergens in asthmatic manifestations.

Regarding the exposure-response relationship, specific bronchial provocation studies clearly show that inhalation of HDM allergens leads to early and, in most patients, late bronchoconstriction and increase in nonspecific BHR [8]. The efficacy of various avoidance measures on many variables has also been demonstrated by several controlled trials in mite-allergic patients with asthma or rhinitis. HDM avoidance measures include: living at altitude (where humidity is lower); use of acaricides to clean carpets and bedding; use of zippered vinyl covers for pillows and mattresses; use of synthetic instead of feather pillows and quilts; removal of bedroom carpets; weekly change of bed linen; twice daily vacuum-cleaning of bed and upholstered furniture; daily cleaning of the bedroom floor; folding back of blankets and upper sheets or duvets to allow mattresses to air; and removal of plants, soft toys, cushions and upholstered furniture from the bedroom. In these trials, the variables studied have included: bronchial provocation tests; asthma symptoms; medication intake; pulmonary function tests; peak expiratory flow; total immunoglobulin (Ig)E; basophil release of mediators; and HDM content in dust samples. All these variables have been found to be improved by avoidance measures [43–48], stressing that even after prolonged exposure, HDM allergens still play a role as triggering factors and as determinants of the severity of asthma. It must be pointed out that studies demonstrating such an efficacy used combinations of avoidance measures rather than one isolated measure; for example, vacuum-cleaning alone is ineffective for mite avoidance, many vacuum-cleaners actually transiently increase airborne mite allergens. This stresses the need for combining multiple anti-mite measures to obtain clinical efficacy.

Regarding the relationship between sensitization and asthma symptoms, studies of animal models of asthma (especially in rodents) have shown that systemic or local sensitization to allergens (such as ovalbumin) can induce airway inflammation and specific bronchial hyperresponsiveness characterized by early- and late-phase bronchoconstriction after allergen challenge [49, 50]. In humans, several clinical trials have studied the effects of conventional subcutaneous immunotherapy with HDM allergens, leading to somewhat contradictory results [6]. These discrepancies are likely to be related to differences in: study design and duration; numbers of patients and selection criteria; modalities of immunotherapy (progression of allergen doses, maintenance doses), and allergen extracts (standardized or not, aqueous, tyrosine-absorbed or alum-precipitated, partially or totally purified).

To clarify these results, nine double-blind, placebo controlled, randomized trials of mite hyposensitization, published between 1966 and 1990, have been included in a recent meta-analysis, which confirms that immunotherapy leads to significant symptomatic improvement and reduction in medication requirement and BHR, and to a small improvement in lung function [51]. To evaluate the risk of overestimation of the efficacy of immunotherapy due to publication bias, the authors calculated that 33 additional negative trials would be needed to overturn these results; so many unpublished studies is unlikely, however. Another source of error is that authors only included published results in their analysis, without studying individual data; this could be a cause of concern, as some negative trials reported incomplete data. Finally, the interaction between efficacy of immunotherapy and concomitant treatment was not studied. Therefore, additional studies are necessary in order to form definite conclusions on this topic, and in particular, to determine the selection criteria of candidates for hyposensitization. Finally, new modalities of immunotherapy are undergoing clinical evaluation, including: systemic immunotherapy with allergen antibody complexes (requiring one hundredth of the amount of allergen used in conventional immunotherapy) [52]; local bronchial hyposensitization (which appears to be limited by safety issues); and oral and sublingual immune therapy [53].

Biological links between HDM and asthma

Immunological and inflammatory responses to HDM allergens

Most adult asthmatics have positive skin tests to HDM. Their immunological anti-*Der p* responses have been extensively characterized *in vitro*. High levels of anti-*Der p* IgE, IgA and IgG are present in 90% of patients who have positive skin tests to *Der p* extracts, whereas, nonsensitized subjects have only low titres of anti-*Der p* IgG [6]. Most studies have found anti-*Der p* IgE only in subjects who also have anti-*Der p* IgG, which is often present in higher titres than IgE. In children, the presence of anti-*Der p* IgG and IgE is correlated with the development of clinical manifestations of atopy (*i.e.* rhinitis, asthma or eczema). In adults who have developed antibodies, although a long-term decrease in their level is observed during life, there is no evidence that this decrease is associated with any improvement in symptoms. Similarly, the correlation between the level of antibodies and the severity of symptoms is poor, both at baseline and during effective immunotherapy. However, mean IgE levels are higher in asthmatic than in rhinitic patients.

With respect to immunoglobulin production, which is thought to be dependent on T-lymphocytes, proliferative T-cell responses to *Der p* are observed in the majority of patients with asthma, atopic dermatitis or allergic rhinitis, who are sensitized to *Der p*, as assessed by skin tests [54, 55]. The proportion of T-cells reactive to *Der p* appears to be higher in patients who have higher titres of anti-*Der p* IgE, as assessed by their RAST class [54, 56]. The characteristics of these T-lymphocytes have

been assessed in several studies: proliferating T-cells are predominantly of the type 2 T-helper (Th2) type (*i.e.* secreting interleukin 4 and 5 (IL-4 and IL-5)), but are not defective in their capacity to produce type 1 T-helper (Th1) cytokines (*i.e.* interleukin 2 (IL-2) and interferon-γ (IFN-γ)) [57–59]; *Der p* specific T-cell clones are heterogeneous regarding their T-cell receptor (TCR) V alpha and beta gene products [58]. Several human leucocyte -DP and -DR (HLA-DP and -DR) restrictions have been described in these clones, differing according to their epitope on the *Der p* molecule [58, 60, 61]. Finally, the magnitude of proliferative T-cell responses to *Der p* is not affected by the release of mediators from basophils [62].

As with other allergens, inhalation of HDM or local bronchial challenge with these allergens has been shown *in vivo* to induce bronchoalveolar and systemic immunological and inflammatory responses in sensitized subjects. Some examples of these responses are: bronchoalveolar recruitment of helper and suppressor T-lymphocytes [63], neutrophils, eosinophils and mast cells [64, 65]; activation of bronchoalveolar macrophages, as assessed by rosette technique or measurement of platelet-activating factor (PAF)-acether and beta-glycuronidase release [9, 65, 66]; activation of eosinophils, which secrete major basic protein, eosinophil cationic protein and neurotoxin [65, 67]; release of histamine recovered in bronchoalveolar lavage fluid (BAL) [65]; elevation of plasma histamine, serum neutrophil chemotactic activity [68], and circulating eosinophils, basophils and their progenitors [69]. Among these features, several differences between early- and late-phase responders have been described: single early responders (*i.e.* patients who do not exhibit any late-phase response) have higher CD8+ titres in BAL, suggesting that these cells prevent the late response [63]; activation of macrophages and eosinophils and recruitment of inflammatory cells is more prominent in late responders [65, 67]; finally, plasma histamine increases are parallel to clinical bronchoconstrictive responses [68].

Direct effects of HDM allergens on the airway epithelium

In addition to immunological mechanisms, HDM allergens may participate in BHR and asthma pathophysiology through other effects: as allergens of *Dermatophagoides* spp. are digestive enzymes of the mite, including a cysteine and several serine proteases [13, 15–21] (table 1), it has been hypothesized that they might directly induce mucosal damage. HERBERT *et al.* [70] recently showed that *Der p* 1 (and mite spent growth medium extract (SGME)) increases bovine bronchial mucosal permeability to serum albumin. They also found that this allergen is capable of causing cell detachment of cultured Madin-Darby canine kidney (MDCK) cells and canine tracheal epithelial cells. Moreover, exposure of the luminal side of bovine bronchial fragments to *Der p* 1 resulted in histological signs of epithelial injury. There was little histological evidence of cytolysis in tissue preparations that were exposed to *Der p* 1 and in which cell detachment was observed, suggesting an effect on cell attachment proteins. The effects observed were potentiated by reducing agents (dithiothreitol and glutathione) and prevented by a cysteine protease inhibitor, L-trans-epoxysuccinyl-leucylamide-(4-guanidino)-butane (E-64), suggesting that they were related to the cysteine protease activity of the allergen.

Similarly, some exogenous serine-proteases have also been shown to damage the epithelium: for instance, elastase from *Pseudomonas aeruginosa* increases paracellular permeability of rat type II pneumocytes (and MDCK cells) by damaging tight junction proteins (ZO-1 and ZO-2), and thereby facilitating transepithelial penetration of macromolecules and bacteria [71]. Such an effect of *Der p* serine proteases has not been demonstrated so far. These data suggest that *Der p* enzymes may participate in the increase in bronchial permeability (assessed by bronchial clearance of radio-labelled diethylenetriamine penta-acetic acid (DTPA)), which has been observed *in vivo* during acute asthma attacks [72]. However, their potency and relevance at their *in vivo* tissue concentrations are not known and have not been compared to that of inflammatory mediators, such as histamine, which has been shown to increase bronchial permeability *in vivo* [73]. *Der p*-induced epithelial cell detachment may also induce inflammatory phenomena by itself, as demonstrated in guinea-pigs, in which *in vivo* epithelial removal results in production of venular gaps and infiltration of neutrophils and eosinophils, the latter being activated [74]. This hypothesis remains to be tested.

HDM enzymes may also affect proteins which are implicated in immunological and inflammatory phenomena. It has recently been demonstrated that *Der p* 1 has not only a cysteine but also a serine protease activity, this mixed enzymatic activity being responsible for cleavage of CD23 (the low-affinity IgE Fc receptor). This cleavage may enhance IgE synthesis by suppressing a normal inhibitory feedback mechanism, and by increasing soluble CD23 (which induces IgE production). This effect is prevented by $alpha_1$-antitrypsin, which may explain how tobacco smoke favours allergic asthma [22].

In addition to their effect on airway epithelial permeability and IgE, the enzymatic properties of *Der p* allergens could participate in the pathogenesis of asthma by directly activating inflammatory cells; such an IgE-independent activation has indeed been described for mast cells [75]. HDM proteases could also directly alter epithelial functions that may have a role in airway inflammation, such as the secretion of inflammatory lipid mediators, oxygen free radicals and cytokines, or the expression of enzymes that inactivate proinflammatory neuropeptides or of major histocompatibility complex (MHC) II and adhesion molecules [76]. Finally, proteolytic activity of *Der p* allergens could alter bronchial secretions by acting (directly or *via* inflammatory responses) either on proteoglycans of the mucus or on airway ion transport [77]. Indeed, a direct effect on the composition of mucus can be hypothesized, as some enzymes, such as mast cell chymase, cathepsin G (a cysteine protease) and neutrophil elastase (a serine protease), are potent secretagogues for airway epithelial mucous cells [78, 79].

However, these hypotheses remain to be demonstrated. In addition, the enzymatic properties of HDM allergens are clearly not sufficient to induce airway sensitization and asthma, since many nonasthmatic subjects are exposed to high levels of HDM.

Relevance of allergen contents and particle sizes of experimental solutions to real life exposures

Even if the above-cited results suggest a direct effect of some *Der p* enzymes on the airway mucosa *in vitro*, they must be evaluated with caution, as they come from studies where the doses of allergen to which epithelial fragments were exposed may be different from those found in a normal environment: HERBERT *et al*. [70] found an effect of *Der p* 1 on cell detachment at concentrations greater than 1 $\mu g \cdot mL^{-1}$. The effect on epithelial permeability to albumin occurred with concentrations of 300 $\mu g \cdot mL^{-1}$ *Der p*. The effect on CD23 reported by HEWITT *et al*. [22] was observed at concentrations greater than 3 $\mu g \cdot mL^{-1}$ *Der p* 1.

Mite faeces contains up to 10 mg *Der p* $1 \cdot mL^{-1}$ [80]. Each faecal particle contains approximately 100 μg *Der p* 1. During normal domestic activity, a filter that samples air for 45 min at 17 $L \cdot min^{-1}$ collects 1–30 ng of *Der p* 1 [80]. The level of airborne allergen actually depends on the degree of disturbance of the room in which measures are performed (ranging from 30 $pg \cdot m^{-3}$ in undisturbed rooms to 30 or even 90 $ng \cdot m^{-3}$ after disturbance), and on the concentration of allergen in reservoirs, such as mattresses and carpets (*i.e*. 1–200 $\mu g \cdot g^{-1}$ of dust) [8]. Moreover, the quantity of inhaled allergen is likely to increase during close contact with these reservoirs, *e.g*. during sleep.

Eighty percent of inhaled allergens are contained in faecal particles, which have a diameter of more than 10 μm [80, 81], which is the usual threshold for penetration in the lower respiratory tract. However, it has been shown that 13% of particles of 16 μm can deposit in the human airways [82]. Once in the airways, allergens elute from these particles and very high local concentrations are thought to be reached in some areas (10 $mg \cdot mL^{-1}$ *Der p* 1 [8]. The cumulative doses of allergen that reach lower airways in environmentally-exposed subjects are difficult to assess. FERGUSON and BROIDE [83], found concentrations of 3.4±1.0 ng of *Der p* 1 per mL of 20 fold concentrated BAL fluid in sensitized subjects with rhinitis and/or asthma who were exposed to 6–27 μg immunoreactive *Der p* $1 \cdot g^{-1}$ of dust. To summarize these data, it can be hypothesized that normal exposure leads to very high concentrations of HDM allergens in limited areas of the airways, whilst prolonged exposure results in cumulative doses of several $\mu g \cdot week^{-1}$.

During specific bronchial provocation challenges, inhaled concentrations are higher (*e.g* 10–100 $mg \cdot mL^{-1}$) and particle diameters are lower (2 μm) than during natural exposure, but the duration of exposure is very short (1–2 min), leading to cumulative quantities which may be compared to what is obtained after several days or weeks of natural exposure [6, 8, 66, 69]. During segmental challenges, lower concentrations (200 $ng \cdot mL^{-1}$ *Der p*) induce local inflammatory responses [9]. Thus, concentrations used in *in vitro* studies or in bronchial challenges may not be out of proportion when compared to *in vivo* natural exposure. However, the time course of airway exposure to HDM allergens is obviously different; moreover, the amount of allergen effectively reaching epithelial cells *in vivo* may be limited by trapping of these allergens in the mucous layer.

In conclusion, epidemiological data have shown that early exposure to HDM is a risk factor for asthma, and persistent exposure to high levels of allergens leads to symptoms and increases asthma severity. HDM allergens are actually among the most potent determinants of asthma, even though they are obviously not the only causative factor in this disease (many asthma cases occur in non-HDM-sensitized subjects, and a causal role of other allergens, such as cat allergens, is also suspected). Clinical experimental data confirm the role of sensitization to HDM in the development and persistence of the disease, and the role of HDM allergens as triggering factors of asthma symptoms. The causative role of HDM in the pathogenesis of asthma may relate not only to proinflammatory immunological responses but also to direct epithelial alterations, due to the enzymatic properties of these allergens; however, the *in vivo* relevance of these enzymatic effects is still hypothetical.

Even if the occurrence and severity of asthma is likely to depend on a combination of the level of personal exposure to house dust mites and other individual environmental or genetic risk factors, these observations support the recommendations for house dust mite avoidance in sensitized subjects and, possibly, in children who are at high risk of developing asthma.

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