

.....

Modeling Responses to Respiratory House Dust Mite Exposure

*Elizabeth C. Cates, Ramzi Fattouh, Jill R. Johnson,
Alba Llop-Guevara, Manel Jordana*

Department of Pathology and Molecular Medicine, Division of Respiratory Diseases and Allergy, Centre for Gene Therapeutics, McMaster University, Hamilton, Ont., Canada

Abstract

House dust mite (HDM) is the most pervasive indoor aeroallergen source worldwide. Allergens derived from HDM are associated with sensitization and allergic asthma. Allergic asthma is an immunologically driven disease characterized by a Th2-polarized immune response, eosinophilic inflammation, airway hyperreactivity, and remodeling. Animal models of asthma utilizing ovalbumin (OVA) exposure have afforded us considerable insight with respect to the mediators and cell types involved in allergic airway inflammation. However, OVA preparations and HDM are two vastly different materials. This chapter is specifically concerned with modeling responses to HDM exposure in mice. These studies have furnished new information and unlocked new lines of inquiry regarding biological responses to common aeroallergens. The complexity of HDM as an allergen source, with its plethora of protein and nonprotein immunogenic components, may influence the mechanisms underlying sensitization, inflammation and remodeling. Here, we will discuss this issue, along with giving critical thought to the use of experimental models.

Copyright © 2007 S. Karger AG, Basel

Introduction

Allergic asthma is now understood as an immunologically driven disease that occurs in predisposed individuals as a consequence of aeroallergen exposure. The hallmarks of the asthmatic phenotype principally include the development of a Th2-polarized immune response, eosinophilic inflammation and airway hyperreactivity (AHR); persistent or recurrent inflammation appears to be associated with changes in the structure of the airway referred to as remodeling. This chapter is specifically concerned with modeling responses to exposure

to house dust mite (HDM), the most pervasive aeroallergen worldwide. Throughout the chapter, our hope is to place our discussion in the broader context of modeling of asthma, beginning with a foreword about scientific modeling.

Modeling is an essential part of the scientific endeavor. In medicine, illness is manifested by a collection of signs and symptoms. An observer collects them, appraises them and decides to which recognized pattern they best fit. This pattern recognition exercise, essential in deriving a diagnosis, contributes only modestly to the understanding of the origin of a disease, its evolution and its nature. Origin, evolution and nature are facets of a complex process, especially in the case of chronic diseases such as asthma. Advancing our understanding of these facets will come not only from a thorough study of the literature and much careful consideration but, much more likely, through experimentation. Indeed, it has been largely through experimentation in *in vitro* systems as well as in *in vivo* animal and human models that we have learned the most about asthma.

Biomedical modeling generally differs from modeling in other scientific domains. In disciplines such as mathematics, physics and economics, a model is the elaboration of a set of rules governing interactions between different variables. Thus, by definition, there is not a predetermined outcome. For example, the equation that defines Newton's Second Law of Motion, $F = ma$ where ' F ' is the force applied, ' m ' the mass of the object and ' a ' is acceleration, is such a model. These kinds of models are particularly suited to explain and predict the outcome of interactions between a number of variables. In sharp contrast, the outcomes of biomedical models are predetermined; in other words, their goal is to recapitulate a given phenotype. The implication of this is that *one can only model what one knows*. This is what determines the logic of biomedical modeling. The undisputed, but somewhat neglected, corollary of this is that modeling of a disease should be a dynamic and ever-evolving endeavor. An underlying theme of this chapter is that modeling promotes questioning which, in turn, echoes the stage of knowledge at a given point and time.

The notion that one can only model what one knows underlies the development of models of asthma over the last 20–30 years. Inflammation, or features of inflammation, had been noted for a long time in asthmatic individuals. However, the appreciation that eosinophilic airway inflammation resulting from a particular type of immunological reaction is a defining feature of the asthmatic phenotype is more contemporary. This understanding, in conjunction with the explosion of molecular immunology, steered the modeling strategies that researchers chose to explore in recapitulating the asthmatic phenotype, particularly in mice. The benefits of this work are apparent as it is now established that allergic asthma is the product of a Th2-polarized response with all of the attendant implications, including the discovery of the functions of archetypical

Th2 cytokines, notably IL-4, IL-5, IL-9 and IL-13, on cardinal features of the asthmatic phenotype.

The vast majority of research in models of asthma to date has utilized chicken egg ovalbumin (OVA), an innocuous protein, as a surrogate allergen. It appears that early research in anaphylaxis led investigators to make this choice, as there is historical evidence that experimental research in anaphylaxis, notably to food antigens, considerably preceded experimental research in asthma. Indeed, studies of OVA-induced anaphylaxis in animal models date back nearly 100 years [1, 2]. Research into these two processes evolved rather separately for a long period of time. It seems plausible that the realization of the conceptual and mechanistic connections between anaphylaxis and asthma beginning with Meltzer in 1910 [3], along with the easy availability and very low cost of OVA, set the stage for researchers wishing to model asthma to settle on OVA as the ‘pseudo-allergen’ of choice.

Mouse Models of Allergic Airway Disease

OVA-Based Models of Asthma

There have been many studies, particularly over the last 20 years, in experimental models of asthma. The objective of these initiatives has been to recapitulate the cardinal features of the asthmatic phenotype, notably airway inflammation but also AHR and, more recently, airway remodeling. As indicated earlier, most of the research in this field has been performed in models using OVA, a choice that has fundamentally influenced the strategies employed to generate allergic sensitization. Indeed, the fact that exposure to OVA, either intranasally or by aerosol, to naïve mice elicits inhalation tolerance rather than allergic sensitization or airway inflammation [4–7] has forced researchers to develop ways of circumventing inhalation tolerance. With some minor variations, the most commonly used strategy involves the adsorption of OVA to a chemical adjuvant (usually aluminum hydroxide or alum) that is injected into the peritoneal cavity to generate allergic sensitization; we will refer to these models as ‘conventional’ models of allergic sensitization from here on. An asthma-like phenotype is, then, elicited by a subsequent airway challenge with OVA. The principal utility of these models resides in the fact that they can generate a consistent and robust inflammatory response and, consequently, allow for the study of the characteristics of such a response. Their greatest limitation is that they are inherently designed to chemically bypass the natural requirements for allergic sensitization, precluding the study of that phenomenon. In addition, neglecting the route by which humans are initially exposed to

aeroallergens – the respiratory mucosa – may have a number of significant immunological consequences.

That allergic asthma is a result of the interaction between inhaled aeroallergens and the immune system brings to the fore the merit of experimental models capable of generating the asthmatic phenotype by exposure through the respiratory route. It seems intuitive that mucosal exposure to OVA, whether via the respiratory or gastrointestinal mucosa, would result in tolerance for there is neither survival advantage nor immunological parsimony in generating a productive immune-inflammatory response against an innocuous protein. A model system was described in which mice were exposed to aerosolized OVA for 10 consecutive days, leading to their sensitization [8]. However, this has not been the finding in most cases. It seems to follow that the lack of inherent immunogenicity of an antigen can be amended by altering the immune status of the host, particularly through the activation of the surveillance apparatus that first interacts with antigens. For example, studies in which granulocyte macrophage colony-stimulating factor (GM-CSF) was overexpressed in the airway milieu enlighten this point because GM-CSF is one of the most powerful natural adjuvants with substantial effects on the maturation and activation of macrophages and dendritic cells. Indeed, it was shown by Stämpfli et al. [9] that GM-CSF enrichment of the airway microenvironment inhibits tolerance induction to OVA and elicits a phenotype with the typical hallmarks of asthmatic inflammation. Mice subjected to 10 OVA aerosolizations in the context of a GM-CSF-enriched microenvironment showed an expansion and activation of antigen-presenting cells (APCs), developed an OVA-specific Th2-polarized immune response associated with airway eosinophilic inflammation and goblet cell hyperplasia, and exhibited AHR. It is likely that molecules other than GM-CSF, such as thymic stromal lymphopoietin, have a direct role in the generation of allergic immune responses [10, 11].

A series of subsequent studies demonstrated that airway overexpression of several cytokines and chemokines such as IL-12, IL-10 and IP-10 were capable of generating distinct immune-inflammatory phenotypes to the same antigen, OVA [12–14]. Together, these studies illustrate that the *context* in which the immune system first sees antigen is of preeminent relevance to the generation of future immune responses and established experimentally, as stated by Lee and Lee [15], the ‘macroimportance of the microenvironment’. Yet this line of experimentation was limited, as it did not account for the fact that common allergens are fundamentally different from OVA, and that humans are rarely ever exposed solely to allergens, or to purified allergenic proteins but rather to complex materials. In other words, the biochemical and presumably immunogenic differences between OVA and the allergenic matter humans inhale, such as HDM, may impact not only the outcome of allergen exposure but also the mechanisms by which these outcomes are brought about.

HDM as Allergen

The discovery of *Dermatophagoides pteronyssinus* as the source of the long-sought ‘house dust allergen’ in 1967 [16] sparked an exponential growth in investigations into the roles of HDM in allergy [17]. Dust mites are ubiquitous throughout humid areas of the world [18]. Of the phylum Arthropoda and class Arachnida, mites are more closely related to spiders, scorpions and horseshoe crabs than they are to insects, and are therefore difficult to control using routine pest control methods. The small size of many mite species (e.g. 20–320 μm for *D. pteronyssinus*, depending on developmental stage) further complicates control of mite populations, all but ensuring exposure. Thirteen different species of mites can be found in house dust, but the three most common species worldwide, and the major sources of mite allergen, are *D. pteronyssinus*, *D. farinae*, and *Euroglyphus maynei*. In tropical climates, *Blomia tropicalis* is also highly prevalent [19]. The discovery of the allergenic role of mites and an understanding of their biology has allowed researchers to address many questions concerning the global distribution of dust allergies, the seasonality of the disease, and the risk associated with damp houses. Many of these issues are still being investigated today.

It has been argued, based on the global prevalence of mite allergens, and the high proportion of total IgE directed towards them, that there is more specific IgE to mite allergens than any other single allergen source in the world [17]. Sensitization to allergens derived from HDM is also strongly associated with allergic asthma, further emphasizing the clinical importance of this allergen source. Much of the knowledge we have in support of which HDM proteins constitute ‘allergens’ comes from the binding of patient IgE in Western blotting studies. However, these studies are difficult to interpret due to the variation between reports, the potential for cross-reactivity of antibodies, and the lability of antigens [reviewed in 20]. Therefore, identifying which HDM components are the most universally relevant to human disease, and developing appropriate models in which to test hypotheses surrounding HDM allergy, add additional layers of complexity.

Domestic mites are complex organisms that produce thousands of different proteins and other macromolecules. The identification and isolation of mites from dust and their subsequent culture laid the foundation for the development of the extracts currently used in research. HDM extracts are made from an aqueous solution of a variable mixture of whole mites, nymphs, fecal pellets, eggs and spent culture media, all of which are relevant sources of allergenic proteins [21, 22]. Based on a study of these extracts and the high concentrations of these allergens in dust, it is believed that the group 1 and group 2 HDM allergens are ‘the major’ allergens. However, it is unknown how well these mite extracts actually compare to the allergens present in the environment. It is

possible that some mite proteins present in house dust are poorly represented in extracts due to, for example, their hydrophobicity, stability and/or ability to withstand the extraction process. In addition, extracts differ based on production methods [23], and are difficult to standardize based on the number of proteins they contain [20].

The advent of molecular biology has allowed for the identification, cloning, and synthesis of the proteins identified as HDM allergens. These developments provided the opportunity to investigate the effects of isolated native proteins or recombinant peptides in allergic asthma to HDM. While this approach permits investigation into the effects of a single agent, there are also some shortcomings. First, some recombinant proteins do not retain potentially important native biological activities after being synthesized without a chemical treatment that may affect the protein in other ways. Also, that exogenous adjuvants are required to elicit allergic sensitization to these individual proteins precludes investigation into the immunological mechanisms that underlie this process. In addition, humans are not exposed to a single protein, but to a complex material made up of a multitude of molecules that have the potential to interact. Thus, experiments utilizing a single HDM protein may not capture the complexity of this ‘material’. On the other hand, HDM and its extracts are complex; therefore, clarifying the functions of their individual active components and how they contribute to immune responses is a challenging endeavor. This dilemma will likely be mitigated by the convergence of progress arising from the mindful exploration of each experimental paradigm.

Acute HDM Exposure Models

Despite the evidently important contribution of HDM proteins to the myriad of allergens that humans are exposed to, relatively little research has been conducted in animal models to discern the nature of the effects of HDM *in vivo*. To date, the vast majority of this work has been done using the previously mentioned ‘conventional’ [intraperitoneal (i.p.) + alum] models of allergic sensitization. Shortly following the discovery of mites as the allergenic source of house dust, and the establishment of culture systems from which to extract mite proteins, research logically focused on the ability to elicit IgE responses in mice and guinea pigs [24–27]. Initially it was discovered that it is possible to sensitize guinea pigs to HDM extracts via the respiratory mucosa [26]. At this time however, sensitization in the absence of adjuvant appeared to be arduous, taking as long as 17 weeks to achieve [24]. In mice, multiple injections of HDM seemed to be required, even in the presence of alum, to generate an IgE response [27, 28]. These early findings appear to have set the stage for the primary use of conventional models of allergic sensitization as the standard in the field. This is true for the majority of studies that have followed, modeling both

allergic asthma and atopic dermatitis, unless modified animals such as T cell receptor transgenics [29] were employed.

Research investigating the immunological mechanisms involved in allergy to HDM began in earnest in the late 1980s to early 1990s. Experiments addressing the allergenic components of HDM extracts have demonstrated that while the higher molecular weight fraction of these extracts (containing Der p 1) does appear to retain the majority of the allergenic potential [30], Der p 1 alone induces oral and inhalation tolerance [31–33]. It is plausible that this finding perpetuated the notion that conventional models were needed to examine the induction of responses to HDM, and the continued use of i.p. models sparked two lines of questioning. One line is concerned with the development models of mucosal sensitization that are more clinically relevant [29, 34, 35], and the other asks immunological questions using conventional i.p. models and the inflammatory responses they elicit. Studies in this latter category have been useful in addressing questions such as the role of eosinophils in AHR elicited by HDM [36], the types of inflammatory responses elicited by different species of mites [37], the role of the proteolytic activity of Der p 1 in inflammatory responses in the lung [38, 39], the importance of the spatial distribution of immune cells in the lungs of primates [40], as well as the feasibility of cytokine gene therapy-based strategies or parasitic infections to treat HDM allergy [41, 42].

As previously mentioned, early attempts to sensitize mice to HDM via the respiratory mucosa were met with limited and inconsistent success. In 1999, Yu et al. [43] demonstrated that sensitization to a crude *D. farinae* extract was possible via the intratracheal route in the absence of exogenous adjuvants. However, the inflammatory responses in the lung and IgE titers obtained were admittedly low, and much more robust responses could be achieved via conventional i.p. sensitization followed by airway challenge. This was followed in 2004 by Cates et al. [44] who published an acute model of allergic sensitization to an HDM extract (*D. pteronyssinus*) in which mice were indeed sensitized via the respiratory mucosa in the absence of exogenous adjuvants. This model was characterized by the current hallmarks of allergic asthma: the expansion and activation of APCs, an eosinophilic inflammation in the lung, deployment of a Th2-polarized immune response, Th2-associated immunopathology and resultant AHR. This acute HDM exposure model also allowed for a preliminary investigation into the roles of a cytokine, GM-CSF, in the early stages of allergic sensitization. In an interesting parallel to the previously reported findings of Stämpfli et al. [9], who demonstrated that enriching the airway environment with GM-CSF allowed for sensitization to OVA, the administration of an anti-GM-CSF antibody in the Cates et al. study markedly decreased responses to HDM. This was believed to be due to the ability of HDM to elicit GM-CSF

production from bronchial epithelial cells [45] and the powerful effects this cytokine has on APCs [reviewed in 46]. Therefore, unlike OVA, HDM appeared to have an intrinsic ability to elicit an immune response, the nature of which can now be studied in more detail. Using a slightly modified model, others later reported similar findings, further expanding their work to examine the impact of regulatory T cells on dendritic cells and allergic responses to HDM [47].

One of the fundamental differences between the models discussed above and allergen exposure in humans is that these animals were exposed to HDM antigens for short, experimentally defined periods of time, whereas human exposure is a ubiquitous life-long event. As such, these kinds of models are inadequate to examine questions surrounding the biological processes that occur as a result of chronic allergen exposure, and if or how these processes play a role in diseases such as allergic asthma.

Modeling the Chronic Aspects of Allergic Asthma

That human exposure to HDM is a chronic event implies that there is a persistent, ongoing interaction between HDM antigens and the immune system. However, the details of the consequences of this interaction in allergic and non-allergic persons are unknown. Currently, it is believed that chronic exposure to allergen results in persistent eosinophilic inflammation, which leads to the structural changes, characteristic of asthma, known as airway remodeling.

Remodeling of the airways was first described quantitatively in 1922 by Huber and Koessler [48], and many subsequent studies have shown this phenomenon to be comprised of increases in mucous production, subepithelial extracellular matrix deposition, and airway contractile elements such as airway smooth muscle cells and myofibroblasts. As the clinical significance of these structural changes to the airway wall has become apparent, efforts have been made to develop model systems to investigate immunological links to this phenomenon.

Many cell types and mediators associated with Th2-polarized immune-inflammatory responses have been implicated, primarily in vitro, in the elaboration of airway structural changes. Cytokines such as IL-13 and IL-4 have been shown to directly effect airway structural cells, leading to increased mucous production [49] and collagen deposition [50], as well as phenotypic changes in fibroblasts [51]. Activated Th2-polarized lymphocytes have also been directly implicated in the increased proliferative capacity of airway smooth muscle cells [52]. Moreover, recent studies into the structural consequences of eosinophil degranulation in the airway have revealed that eosinophil-derived cationic proteins are able to modify gene expression in bronchial epithelial cells, inducing them to synthesize numerous mediators, such as growth factors and matrix metalloproteinases, which ultimately lead to altered extracellular matrix composition and turnover [53]. In addition to the direct impact of inflammatory cells on

airway structure, resident structural cells themselves, including epithelial cells, smooth muscle cells, fibroblasts and myofibroblasts [reviewed in 54], may participate in the initiation of a repair phenotype characterized by increased collagen deposition, which is thought to be driven by TGF- β and other growth factors [55]. Due to the complexity of remodeling, and the number of cell types and mediators involved, the extent and nature of the contributions made by inflammatory and structural cells in airway remodeling and the asthmatic phenotype as a result of chronic allergen exposure *in vivo* remains to be elucidated.

To address these questions, significant effort has been exerted to recapitulate the chronic aspects of asthma, using animal model systems. One approach to investigating the role of Th2-associated molecules in airway remodeling has been the genetic manipulation of the host. By either upregulating pulmonary expression of key Th2 cytokines such as IL-9 and IL-13 [56, 57] or by knocking out expression of t-bet [58], a transcription factor associated with the generation of Th1 immune responses, chronic airway inflammation, peribronchial fibrosis and abnormal airway function can be established. While these models illuminate the potential role of each of these factors in the structural changes associated with asthma, they ignore the potential contribution of allergen exposure itself on the development and maintenance of abnormal airway structure and function.

Acute protocols employing short-term OVA delivery, even at a high dose, have been used to examine the impact of allergen exposure on airway remodeling. However, these protocols do not lead to most of the structural changes associated with chronic asthma. Therefore, the effects of chronic pulmonary exposure to OVA were explored. Interestingly, exposure under these conditions led to an abrogation of the immune-inflammatory response to OVA. Although initial delivery of OVA results in immune activation, sustained respiratory exposure leads to an attenuation of the immune response to OVA, deviation of the immunoglobulin profile from IgE to IgG1, decreased expression of costimulatory molecules on lung mononuclear cells, and decreased eosinophilia in the peripheral blood and bronchioalveolar lavage, even in *i.p.* sensitized animals [59].

In order to avoid the development of inhalation tolerance to OVA, various models have been developed in which OVA is delivered intermittently after *i.p.* sensitization [60–63]. The resultant phenotype of these models includes epithelial lesions (goblet cell hyperplasia, mucous production and epithelial shedding) and alterations to the subepithelium, most notably increased collagen deposition and accumulation of contractile elements (smooth muscle hypertrophy/hyperplasia and myofibroblast differentiation). Many of these models are also associated with increased AHR to methacholine. Interestingly, the extent of eosinophilia subsides progressively with each exposure. In addition, interactions

between many common aeroallergens and the lung microenvironment are a potentially important pathway in the development of altered airway wall structure. This is an aspect that cannot be addressed using OVA as the antigen.

To this effect, models employing chronic exposure to HDM were developed. Recently, in the absence of any adjuvant, chronic exposure to a *D. farinae* extract has been reported to induce airway eosinophilia in infant monkeys [64]. Subsequently, in 2004, Johnson et al. [65] described a mouse model of sustained allergic airway inflammation and remodeling employing chronic, sustained respiratory delivery of a *D. pteronyssinus* extract. This protocol of chronic respiratory exposure to HDM led to sustained airway eosinophilic inflammation, which reached a plateau following 3 weeks of exposure. HDM delivery was associated with immune activation in the lung, characterized by the expansion of APC, activated T cell, and Th2 effector cell populations following 5 weeks of continuous HDM exposure. An allergen-specific memory response to HDM extract was also observed by elevated in vitro production of IL-4, IL-5 and IL-13 by splenocytes isolated from mice chronically exposed to HDM, and humoral immunity to HDM was indicated by elevated serum levels of IgE and HDM-specific IgG₁.

This chronic eosinophilic immune-inflammatory response was also accompanied by profound changes to the structure of the large airways. In addition to the goblet cell hyperplasia and increased mucous production commonly observed in acute allergen exposure protocols, continuous HDM exposure resulted in an increased expansion of peribronchial contractile elements, presumably myofibroblasts, as well as collagen deposition in the airway wall subepithelium. Finally, chronic exposure to HDM had profound functional consequences, with markedly increased AHR to methacholine. Interestingly, the lung structural changes and dysfunction remained following HDM withdrawal, despite an attenuation of the inflammatory response. This suggests that the airway dysfunction seen in this model is contributed to by both airway inflammation and remodeling. It is hoped that a detailed exploration of the evolution of the structural and functional abnormalities in this model will help to improve our understanding of the mechanisms contributing to airway remodeling and dysfunction in the context of chronic exposure to a common aeroallergen.

Considering the Allergenicity of HDM

Clearly, allergic sensitization to a variety of allergens, including OVA and HDM, can be achieved experimentally. These systems do not inform matters such as: (1) what makes allergens like HDM allergenic and (2) under what conditions allergic sensitization naturally occurs. Confronting such questions is the

selective pressure that has driven past changes in modeling and will drive future lines of inquiry.

The Biochemical Nature of HDM Extracts

As previously mentioned, OVA preparations and HDM extracts are, from the perspective of biochemical diversity, two vastly different materials. The OVA preparations that are commonly used in models of allergic disease are typically >98% pure and hence, are essentially free from additional protein and nonprotein components. In stark contrast, HDM extracts are complex materials consisting of hundreds of protein and nonprotein components that range in both size and function. Indeed, it is this fundamental difference between OVA and HDM that is thought to account for the divergent immunological outcomes which arise following inhalation of these materials. This realization has fueled investigations aimed at characterizing HDM-derived allergens and understanding the mechanisms by which these individual components promote allergic disease.

It is estimated that mites produce approximately 3,000 proteins, 5% of which are thought to be allergens [66]. Studies conducted in the mid-late 1980s documented >30 IgE-binding proteins in dust mite extracts using sera from mite-allergic patients [67–69]; however, the actual number of *different* mite allergens represented in those studies may be somewhat less. According to the listing maintained by the Allergen Nomenclature Sub-Committee of the International Union of Immunological Societies [70], 21 different groups of dust mite allergens have been identified. A biological function has been described for most of these allergens, and collectively, they encompass a broad range of functions including binding proteins, structural proteins, enzyme inhibitors and various enzymes [thoroughly reviewed in 20].

The majority of investigations into the impact of HDM-derived allergens on the pathogenesis of allergic disease have focused on the proteolytic activity of HDM proteins. There are at least 4 HDM allergens that possess proteolytic activity. The group 1 allergens display a mixed cysteine/serine protease activity [71], while groups 3, 6 and 9 are serine proteases [72–74] and a number of mechanisms have been identified by which the proteolytic activity of these allergens is thought to contribute to HDM's allergenicity.

A series of *in vitro* studies conducted by Wan et al. [75, 76] using solubilized Der p 1 demonstrated that this protease disturbed a monolayer of cultured bronchial epithelial cells, resulting in minor exfoliation and the disruption of tight junctions. Interestingly, this event was associated with increased epithelial cell permeability and transepithelial migration of Der p 1. Similar effects were observed when bronchial epithelial cells were treated with culture medium enriched in serine proteases derived from *D. pteronyssinus* (i.e. rich in Der p 3, 6

and 9) [77]. Together, these results suggest that the proteolytic activity of HDM may facilitate allergen entry into the submucosal environment. Consequently, this may increase HDM's contact with APCs and the likelihood of developing allergic sensitization [76].

In addition to enhanced epithelial permeability, it has become increasingly clear that the proteolytic activity of HDM-derived allergens can have more direct proinflammatory effects. Alveolar macrophages treated with a *D. farinae* extract in vitro respond by producing IL-6, TNF- α and nitric oxide [78]. Similarly, purified Der p 1, 3 and 9 were collectively shown to induce the production of GM-CSF, IL-6, IL-8 and eotaxin from bronchial epithelial cell lines and primary cultures of human bronchial epithelium [45, 79]. Although the precise mechanism of cytokine induction from mite allergen-treated epithelial cells differs depending on the allergen and cytokine, for the most part these responses seem to be mediated by the activation of protease-activated receptor-2 [79, 80]. More recently, a protease-independent mechanism of Der p 1-mediated cytokine release has been reported [81, 82]. Notably, an HDM allergen lacking any known proteolytic activity (Der p 5) was shown to induce cytokine expression from epithelial cells [82]. Taken together, the increased accessibility of allergens resulting from disrupted epithelial integrity, and a proinflammatory microenvironment may strongly promote the development of allergic sensitization. However, it is important to note that the extent to which these events occur in vivo remains unclear.

It has also been suggested that HDM's proteolytic activity may enhance IgE synthesis as Der p 1, in vitro, can cleave the low-affinity IgE Fc receptor (CD23) from the surface of human B cell lines [83, 84]. CD23 is a key regulator of the IgE network as its binding of IgE and IgE-containing immune complexes on the surface of B cells triggers a negative-feedback signal that prevents further IgE synthesis [85, 86]. Moreover, soluble CD23 has been previously reported to enhance IgE synthesis [87–89]. Thus, it has been proposed that Der p 1-mediated cleavage of CD23 may not only impair IgE feedback inhibition pathways but may also augment IgE synthesis directly [83]. Additionally, both HDM extracts and purified Der p 1 can induce the production of IL-4 directly from mast cells and basophils [90], and this may also enhance the IgE response.

The proteolytic activity of dust mite allergens may, furthermore, bias the development of a Th2 response by cleaving the α -subunit of the IL-2 receptor (CD25) on T cells [91]. Schulz et al. [91] have demonstrated that Der p 1-mediated cleavage of CD25 from human peripheral blood lymphocytes resulted in decreased proliferative and cytokine (IFN- γ) responses upon stimulation with anti-CD3 antibody in vitro. Subsequently they demonstrated that Der p 1-conditioned human CD4 and CD8 T cells produce more IL-4, and less IFN- γ ,

following stimulation with anti-CD3 *in vitro* than T cells treated with inactivated Der p 1 [92]. These findings suggested that HDM may limit the development of Th1 response, at the same time promoting the development of a Th2 response and IgE synthesis [91]. Importantly, this notion is supported by *in vivo* evidence showing that mice immunized with proteolytically active Der p 1 generate higher titers of Der p 1-specific IgE compared to controls immunized with inactivated Der p 1 [93].

The recent discovery of the involvement of chitinases in the pathogenesis of experimentally induced allergic airway inflammation may have uncovered another mechanism by which the functions inherent to HDM proteins may contribute to their allergenicity [94]. Chitinases are enzymes that catalyze the hydrolysis of chitin and are found in arthropods such as dust mites. In addition, a family of chitinase and chitinase-like genes has been identified in both rodents and humans [95–97].

Elias et al. [98] have postulated that some mammalian chitinases may act as potent mediators of the Th2 immune response. In support of this concept, they found increased expression of acidic mammalian chitinase (AMCase) following sensitization and challenge with OVA and, importantly, neutralization of AMCase markedly reduced inflammation and AHR [94]. The expression of mammalian chitinase-like proteins has also been observed in the context of Th2-mediated inflammation during experimental parasitic infection [99]. Moreover, a putative member of the chitinase protein family, ECF-L, may be a potent chemoattractant for eosinophils and T cells [99, 100]. These findings indicate that mammalian chitinases/chitinase-like proteins may play a role in the modulation of the allergic response.

The group 15 and 18 HDM allergens have been identified as chitinases [101, 102]. Based on the findings discussed above, it is reasonable to speculate that the HDM-derived chitinase allergens may function in a manner similar to human chitinase proteins to potentiate Th2 responses. To our knowledge, there is no direct evidence in support of this. However, the group 15 and 18 dust mite allergens show a high frequency of binding to IgE in patient sera, and are the main IgE-binding proteins in allergic dogs [102–104]. This supports the notion that HDM-derived chitinase allergens may influence allergic disease.

Clearly, the functional properties of allergens can profoundly influence their ability to elicit an allergic immune response, although it is likely that in some cases function may be of little significance. For the most part, as it pertains to many of the HDM-derived allergens, this information is simply lacking at this time. For example, the group 10 and 11 allergens have been identified as muscle proteins (tropomyosin and paramyosin, respectively) and have been shown to bind IgE at a high frequency [105, 106]. Similarly, the group 2 allergens bind a high proportion of patient IgE, and have thus been identified as

major allergens. In addition, the group 14 allergens are abundant mite proteins that function as lipid transport or storage molecules [107, 108]. Interestingly, they have demonstrated both potent reactivity in T cell assays and high IgE binding. It remains to be seen if these biochemical functions or immunological activities contribute to HDM's allergenicity.

The complexity of HDM extracts is further enhanced because, in addition to the diverse array of protein allergens, they contain a number of nonprotein components. Of these, lipopolysaccharide (LPS) is the best defined. It is widely acknowledged that LPS is a potent stimulator of the innate immune response and, correspondingly, has powerful adjuvant activity. LPS can elicit the production of a panoply of proinflammatory mediators including cytokines such as TNF- α , GM-CSF, IL-6, IL-8, IL-10 and IL-12 [109, 110], some of which are thought to contribute to the development of allergic responses [110, 111]. That LPS exposure can facilitate allergic sensitization and exacerbate airway inflammation has been demonstrated in experimental systems [112, 113] and moreover, is associated with the severity of allergic airway diseases in humans [110]. Numerous other nonprotein immunostimulators such as lipoteichoic acid and peptidoglycans are abundantly present in the environment, and at least some of these may promote allergic sensitization [114]. However, aside from LPS, very little is known regarding the content of such molecules in HDM. Given their ubiquitous distribution and profound influence on innate immunity, it is likely that at least some of these molecules may also contribute to HDM's inherent allergenicity. Clearly, a comprehensive description of the nonprotein constituents of HDM is required.

It has become apparent that HDM extracts possess, in sharp contrast to OVA, a number of entities capable of distinctly engaging the immune system. What then, could be the immunological relevance of the biochemical differences between OVA and HDM? It seems intuitive that the biochemical and immunogenic profile of HDM extracts will draw on an expanded network of cellular responses and unique molecular signatures. Take for example, the outcome of airway remodeling. While OVA can, under certain conditions (e.g. i.p. sensitization with intermittent exposure) lead to remodeling, achieving the same outcome utilizing an HDM extract and a route of mucosal sensitization does not imply the same underlying biochemical or immunological process; nor can this knowledge be universally applied to the responses elicited by other aeroallergen families. In this regard, preliminary data from our laboratory suggests that in contrast to OVA-based systems [115], TGF- β may *not* be involved in the generation of HDM-induced airway remodeling [116]. So, while some of the experimental outcomes of using OVA or HDM may be quite similar, the mechanistic processes that underlie those outcomes are likely to be fundamentally different.

The Issue of Exposure

A common denominator of the studies cited above is that, in all instances, the concentration of HDM extract administered was experimentally determined by the researchers to achieve their desired outcome (e.g. 25 µg/day/mouse). Therefore, a legitimate question is: how comparable is this concentration of allergen to human exposure? Absolutely the question is intuitive and legitimate; however, the answer is just as elusive. Arguably, the significance of this issue is relative, because *a model of a chronic disease such as allergic asthma is the compression in time and space of a protracted process*. ‘In time’ because the objective is to achieve a predetermined outcome in a relatively short period of time; indeed, the alternative of achieving such a goal in 1–2 years would be a self-defeating enterprise. ‘In space’ because, clearly, ‘real life’ is not the same as life in a cage in an animal facility or, for that matter, the environment in a university research laboratory (in the case of human research). These are the inherent limitations of experimental modeling where the trade-off between ‘real life’ context and the control of variables and productivity must be constantly scrutinized. The relevance of the question of comparative exposure is also dependent on the nature of the overall research question being asked. If the query is: what concentration of, and under what conditions, an HDM extract elicits allergic sensitization and/or airway inflammation, then the information derived from using a single concentration of 25 µg is largely uninformative. Studies comprehensively examining a wide range of doses and lengths of exposure would shed light on these questions. However, if the issue is to consistently establish a robust phenotype with the typical hallmarks of asthma, then, that concentration is suitable. Nevertheless, these comments should not be construed as intellectual trickery to circumvent the genuine relevance of the initial question: how comparable is this experimental dose of allergen to human exposure? The reply is saturated with uncertainty because the terms of reference are ambiguous. Indeed, many clinical and epidemiological studies have measured concentrations of mite allergens. However, the site sampled, the sampling techniques used (type of collectors, frequency of sampling, environment), and the methods of measurement and quantification of allergen employed are diverse. Moreover, the criteria employed to define ‘risk’ (for sensitization as well as disease) are not uniform. Therefore, to precisely identify the concentrations of HDM associated with allergic sensitization and airway disease in humans is an arduous endeavor.

With respect to allergen sampling, there is still debate as to whether *indirect* personal exposure should be assessed by measuring allergen, usually Der p 1, either in airborne dust, in settled dust, or in allergen reservoirs [117]. *Airborne allergen* has been commonly measured by air samplers or ion-charging devices [117, 118]. Under these conditions, allergen is usually expressed as nanograms

per cubic meter of air. Most investigators have been unable to detect airborne mite allergens in the absence of experimental or household disturbance, likely because these levels are below the detection limit for most assays. Allergens in *settled dust* can be detected and measured by leaving Petri dishes out for 2 weeks or using an adhesive-membrane system [117]. In this case, allergen-settling rate is calculated as nanograms per square meter per day. A third method used to measure allergen concentrations is to sample *allergen reservoirs* by means of vacuum sampling [117]. In this instance, exposure to indoor allergens is usually expressed as the concentration of allergen detected in dust samples ($\mu\text{g/g}$ dust); this is considered to be the most consistent method available [119]. Despite controversy as to how the allergen concentrations measured using these varied sampling methods might correlate with each other [117, 120], measurement of mite allergen concentration in dust reservoirs, as a surrogate for airborne exposure, is the most commonly used method.

The site of allergen sampling is also relevant. Mattresses, carpets/floors and upholstered furniture are the best places indoors to detect mites and mite products. Other places with adequate textile substratum and optimal temperature, humidity, and food resources for mites are shutters, clothing and picture frames [121]. It has been proposed that mattress-dust sampling is the most appropriate marker of indoor allergen exposure because bedding is a major reservoir of mite allergens and probably the most important environment for exposure. A number of studies have documented that the amount of mite allergens found in mattresses is up to 10-fold higher than in other areas of a household [122, 123]. It should be noted that the distribution of allergen is dynamic. In particular, the concentration of airborne allergen is notably influenced by any 'disturbance factor'. For example, it has been shown that the airborne levels of Der p 1 increase about 1,000-fold in disturbed conditions, such as bed making [122] or vacuum cleaning done without a filter.

With these considerations in mind, many clinical and epidemiological studies have examined the amount of mite allergen present in homes. The central message that emerges from these studies is that there is an extraordinary geographical variability in terms of both the number of households where mites can be detected and the concentration of mite proteins detected at each site. A recent study by Zock et al. [124] examined over 3,500 households in 22 European cities representing 10 countries. The percentage of households in which mite allergens were detected ranged from 0 to 98.4% (globally, an average of 48.4%). Detection of mite allergens was performed by vacuuming 1 m² of bed for 2 min; under these conditions, the concentration of mite allergens detected (Der p 1 and Der f 1) ranged from 0.01 to 14.71 $\mu\text{g/g}$ of dust. Other European studies have found concentrations of mite allergens ranging from 0.18 $\mu\text{g/g}$ of dust (carpets) and 5.6 $\mu\text{g/g}$ of dust (mattresses) in Germany [125],

to 16 and 28 $\mu\text{g/g}$ of dust in Southern England and Manchester [126, 127], respectively. Leaderer et al. [128] showed that 47% of households in Connecticut and Central Massachusetts had levels of HDM greater than or equal to 2 $\mu\text{g/g}$ of dust, and in 22% of households levels were greater than or equal to 10 $\mu\text{g/g}$ of dust. Higher levels were reported in coastal Australia and New Zealand where prevalence and severity of asthma is the highest [129]; indeed, a study in Wellington reported that at least 2 $\mu\text{g/g}$ of dust were detected in all households, and 36% had levels greater than 100 $\mu\text{g/g}$ of dust [130, 131]. Finally, many studies have shown that there is a plethora of variables influencing the concentration of mite allergens in the household including humidity in particular, temperature, living conditions such as dampness, number of people living in a household and availability of central heating, living habits such as vacuuming mattresses and sleeping with the window open in winter, and many others [119, 124, 132–134]. Some of these variables not only influence the amount of mite allergen that can be detected but also they are likely to alter the actual amount of airborne allergen.

It is, at this point, tempting to furnish an answer to the question of how daily human exposure to mite allergens compares to the daily exposure of mice to these same allergens in experimental systems. The amount of HDM that interacts with the respiratory mucosa of the lower airway of a mouse may be 5- to 10-fold less the amount deposited into its nostrils [135]. Despite this degree of fluctuation, the actual amount of HDM a mouse is experimentally exposed to per day can be reasonably estimated. Clearly, it is far less direct to deduce the amount of allergen interacting with the lower airway respiratory mucosa of a human on a per day basis from the number of micrograms of Der p 1 per gram of collected dust. For example, that several micrograms of Der p 1 can be measured in a sample that has been collected by vacuuming 1 m^2 of a mattress for 2 min says little about how much was made airborne, inspired, and passed the necessary physical barriers to reach the lower respiratory tract. In other words, the logical ambiguity in precisely determining mucosal exposure to HDM in humans precludes the beneficial intent to formulate a rigorous interspecies comparison of exposures.

A different but relevant issue is the relationship between levels of exposure and the risk for sensitization, an altogether different issue to that of the relationship between allergen exposure and allergic disease. While some controversy remains, the consensus is that there is an association between measurable levels of HDM allergen and prevalence and severity of allergic sensitization [136–138]. However, whether this relationship is linear remains to be elucidated. Different studies have provided somewhat different estimates, but it has been put forth that Der p 1 levels as low as 2 $\mu\text{g/g}$ of dust are a significant risk for allergic sensitization [119, 139]. While such measurements are clearly

useful as an epidemiological reference in defined settings, extrapolation of detected amounts of Der p 1 in dust to explaining pathogenesis may extend beyond applicability. That some studies have failed to uncover a relationship between the concentration of mite allergens per gram of dust and the extent of allergic sensitization is not highly surprising, as there may be a number of reasons accounting for this apparent disconnect. For example, humans are not really exposed only to Der p 1, or Der f 1, but to complex materials, which, as previously discussed, contain other immunologically active moieties. Thus, the mite materials humans are exposed to ‘in the wild’ are more than likely to be rather heterogeneous. In addition, immune responsiveness to HDM (or to Der p 1) is surely influenced by the immune status of the host at the time of exposure. For example, concurrent exposure to other immunologically active entities such as molds and viruses, preexisting sensitization to other non-HDM allergens, or exposure to pollution among other things may prime the immune system altering, either way, the threshold of responsiveness to mite allergens. This complex interplay would directly impact on the concentration of HDM (or Der p 1) required to elicit allergic sensitization. In any case, failure to find a correlation between allergen exposure and sensitization does not justify the conclusion that these two variables are not related.

Closing Remarks

Evidently, modeling is pivotal to advancing knowledge in any scientific discipline. In the medical sciences, experimental modeling aims to recapitulate a predetermined outcome. While the generally implied goal of modeling is to ‘mimic a disease’, we must be mindful that this is a misguided enterprise. Overall, disease is an experience that is virtually impossible to duplicate, especially across species. Indeed, the experience and manifestation of disease have not only many anatomical and physiological constraints but also fundamentally distinct social and psychological aspects, all of which have an impact on the immune system. Perhaps, the most constructive way to conceptualize modeling is that it is an attempt to recapitulate the phenotype that we think, *based on our best understanding at a given point in time*, underlies a disease state.

Indeed, the notion of ‘*our best understanding*’ does not refer only to the amount of information available at any point in time. Of course information plays a central role but equally, if not more important, is the current conceptualization, the established paradigm that ‘explains’ the disease. This, we surmise, is what fuels future and novel modeling initiatives. In other words, if asthma is understood, as it was historically, as a ‘nervous imbalance’, or a ‘mediator hyper-release process’, then modeling will go along one path. If, in contrast,

asthma is understood as an immune-driven disease, then modeling will evolve along a very different path, both practically and conceptually.

A number of experimental models of asthma have been developed to date. The past, present and future usefulness of each one of them rests in the ability of researchers to remain both cognizant of the limitations of each experimental construct, and open to the changes that must occur with time and discovery. The greatest liability of exploiting a model uncritically is that it may end up furnishing knowledge alienated from, and hence of doubtful relevance to, the very disease process it was meant to investigate.

Acknowledgements

The authors wish to thank Amal Al-Garawi and Alexander Caudarella for helpful discussion. ECC and RF were the holders of Canada Graduate Scholarships from the CIHR and ECC is the holder of an Eva Eugenia Lillian Cope Research Scholarship. JRJ is the holder a CIHR Doctoral Research Award, AL-G holds a La Caixa Scholarship (Spain) and MJ holds a Senior Canada Research Chair in Immunobiology of Respiratory Diseases and Allergy.

References

- 1 Wells HG: Studies on the chemistry of anaphylaxis. III. Experiments with isolated proteins, especially those of the hen's egg. *J Infect Dis* 1911;9:147.
- 2 Laroche G, Richet C, Saint-Girons F: Anaphylaxie et immunité alimentaires expérimentales, à l'ovalbumine. *C R Soc Biol* 1913;1:87–89.
- 3 McFadden ER Jr: A century of asthma. *Am J Respir Crit Care Med* 2004;170:215–221.
- 4 Holt PG, Batty JE, Turner KJ: Inhibition of specific IgE responses in mice by pre-exposure to inhaled antigen. *Immunology* 1981;42:409–417.
- 5 Swirski FK, Gajewska BU, Alvarez D, Ritz SA, Cundall MJ, Cates EC, Coyle AJ, Gutierrez-Ramos JC, Inman MD, Jordana M, Stämpfli MR: Inhalation of a harmless antigen (ovalbumin) elicits immune activation but divergent immunoglobulin and cytokine activities in mice. *Clin Exp Allergy* 2002;32:411–421.
- 6 Tsitoura DC, Blumenthal RL, Berry G, Dekruyff RH, Umetsu DT: Mechanisms preventing allergen-induced airways hyperreactivity: role of tolerance and immune deviation. *J Allergy Clin Immunol* 2000;106:239–246.
- 7 Tsitoura DC, DeKruyff RH, Lamb JR, Umetsu DT: Intranasal exposure to protein antigen induces immunological tolerance mediated by functionally disabled CD4+ T cells. *J Immunol* 1999;163:2592–2600.
- 8 Hamelmann E, Oshiba A, Loader J, Larsen GL, Gleich G, Lee J, Gelfand EW: Antiinterleukin-5 antibody prevents airway hyperresponsiveness in a murine model of airway sensitization. *Am J Respir Crit Care Med* 1997;155:819–825.
- 9 Stämpfli MR, Wiley RE, Neigh GS, Gajewska BU, Lei XF, Snider DP, Xing Z, Jordana M: GM-CSF transgene expression in the airway allows aerosolized ovalbumin to induce allergic sensitization in mice. *J Clin Invest* 1998;102:1704–1714.
- 10 Al-Shami A, Spolski R, Kelly J, Keane-Myers A, Leonard WJ: A role for TSLP in the development of inflammation in an asthma model. *J Exp Med* 2005;202:829–839.

- 11 Zhou B, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, Lewis DB, Gyarmati D, Aye T, Campbell DJ, Ziegler SF: Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005;6:1047–1053.
- 12 Stämpfli MR, Scott Neigh G, Wiley RE, Cwiartka M, Ritz SA, Hitt MM, Xing Z, Jordana M: Regulation of allergic mucosal sensitization by interleukin-12 gene transfer to the airway. *Am J Respir Cell Mol Biol* 1999;21:317–326.
- 13 Stämpfli MR, Cwiartka M, Gajewska BU, Alvarez D, Ritz SA, Inman MD, Xing Z, Jordana M: Interleukin-10 gene transfer to the airway regulates allergic mucosal sensitization in mice. *Am J Respir Cell Mol Biol* 1999;21:586–596.
- 14 Wiley R, Palmer K, Gajewska B, Stämpfli M, Alvarez D, Coyle A, Gutierrez-Ramos J, Jordana M: Expression of the Th1 chemokine IFN-gamma-inducible protein 10 in the airway alters mucosal allergic sensitization in mice. *J Immunol* 2001;166:2750–2759.
- 15 Lee NA, Lee JJ: The macroimportance of the pulmonary immune microenvironment. *Am J Respir Cell Mol Biol* 1999;21:298–302.
- 16 Voorhorst R, Spieksma FTM, Varekamp H, Leupen MJ, Lyklema AW: The house-dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house-dust allergen. *J Allergy* 1967;39:325–339.
- 17 Tovey E: Effect of Voorhorst's work on the current understanding of the role of house dust mites in allergic diseases. *J Allergy Clin Immunol* 2004;113:577–580.
- 18 Arlian LG: Mites are ubiquitous: are mite allergens, too? *Ann Allergy Asthma Immunol* 2000;85:161–163.
- 19 Arlian LG, Platts-Mills TA: The biology of dust mites and the remediation of mite allergens in allergic disease. *J Allergy Clin Immunol* 2001;107:S406–S413.
- 20 Thomas WR, Smith WA, Hales BJ, Mills KL, O'Brien RM: Characterization and immunobiology of house dust mite allergens. *Int Arch Allergy Immunol* 2002;129:1–18.
- 21 Stewart GA, Butcher A, Lees K, Ackland J: Immunochemical and enzymatic analyses of extracts of the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1986;77:14–24.
- 22 Tovey ER, Chapman MD, Platts-Mills TA: Mite faeces are a major source of house dust allergens. *Nature* 1981;289:592–593.
- 23 Geissler W, Maasch HJ, Winter G, Wahl R: Kinetics of allergen release from house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1986;77:24–31.
- 24 Ishii A, Matsushashi T, Miyamoto J, Sasa M: Production of homocytotropic antibody in guinea pigs inhaled with an extract of the house dust mite, *Dermatophagoides farinae*. *Jpn J Exp Med* 1973;43:25–32.
- 25 Chopra SL, Vincent L: Investigations on alum-precipitated pyridine-extracted allergens. II. Sensitization studies in guinea pigs. *Int Arch Allergy Appl Immunol* 1974;46:321–330.
- 26 Ishii A, Takaoka M, Matsui Y: Production of guinea pig reaginic antibody against the house dust mite extract, *Dermatophagoides pteronyssinus*, without adjuvant. *Jpn J Exp Med* 1977;47: 377–383.
- 27 Kudo K, Okudaira H, Miyamoto T, Nakagawa T, Horiuchi Y: IgE antibody response to mite antigen in the mouse. Suppression of an established IgE antibody response by chemically modified antigen. *J Allergy Clin Immunol* 1978;61:1–9.
- 28 Inagaki N, Tsuruoka N, Goto S, Matsuyama T, Daikoku M, Nagai H, Koda A: Immunoglobulin E antibody production against house dust mite, *Dermatophagoides farinae*, in mice. *J Pharmacobiodyn* 1985;8:958–963.
- 29 Jarman ER, Tan KA, Lamb JR: Transgenic mice expressing the T cell antigen receptor specific for an immunodominant epitope of a major allergen of house dust mite develop an asthmatic phenotype on exposure of the airways to allergen. *Clin Exp Allergy* 2005;35:960–969.
- 30 Tategaki A, Kawamoto S, Okuda T, Aki T, Yasueda H, Suzuki O, Ono K, Shigeta S: A high-molecular-weight mite antigen (HM1) fraction aggravates airway hyperresponsiveness of allergic mice to house dusts and whole mite cultures. *Int Arch Allergy Immunol* 2002;129:204–211.
- 31 Hoyne GF, Callow MG, Kuhlman J, Thomas WR: T-cell lymphokine response to orally administered proteins during priming and unresponsiveness. *Immunology* 1993;78:534–540.
- 32 Hoyne GF, O'Hehir RE, Wraith DC, Thomas WR, Lamb JR: Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. *J Exp Med* 1993;178:1783–1788.

- 33 Stewart GA, Holt PG: Immunogenicity and tolerogenicity of a major house dust mite allergen, Der p 1 from *Dermatophagoides pteronyssinus*, in mice and rats. *Int Arch Allergy Appl Immunol* 1987;83:44–51.
- 34 Duez C, Tsicopoulos A, Janin A, Tillie-Leblond I, Thyphronitis G, Marquillies P, Hamid Q, Wallaert B, Tonnel AB, Pestel J: An in vivo model of allergic inflammation: pulmonary human cell infiltrate in allergen-challenged allergic hu-SCID mice. *Eur J Immunol* 1996;26:1088–1093.
- 35 Herz U, Schnoy N, Borelli S, Weigl L, Kasbohrer U, Daser A, Wahn U, Kottgen E, Renz H: A human-SCID mouse model for allergic immune response bacterial superantigen enhances skin inflammation and suppresses IgE production. *J Invest Dermatol* 1998;110:224–231.
- 36 Tournoy KG, Kips JC, Schou C, Pauwels RA: Airway eosinophilia is not a requirement for allergen-induced airway hyperresponsiveness. *Clin Exp Allergy* 2000;30:79–85.
- 37 Carvalho AF, Fusaro AE, Oliveira CR, Brito CA, Duarte AJ, Sato MN: *Blomia tropicalis* and *Dermatophagoides pteronyssinus* mites evoke distinct patterns of airway cellular influx in type I hypersensitivity murine model. *J Clin Immunol* 2004;24:533–541.
- 38 Gough L, Campbell E, Bayley D, Van Heeke G, Shakib F: Proteolytic activity of the house dust mite allergen Der p 1 enhances allergenicity in a mouse inhalation model. *Clin Exp Allergy* 2003;33:1159–1163.
- 39 Kikuchi Y, Takai T, Kuhara T, Ota M, Kato T, Hatanaka H, Ichikawa S, Tokura T, Akiba H, Mitsuishi K, Ikeda S, Okumura K, Ogawa H: Crucial commitment of proteolytic activity of a purified recombinant major house dust mite allergen Der p1 to sensitization toward IgE and IgG responses. *J Immunol* 2006;177:1609–1617.
- 40 Miller LA, Hurst SD, Coffman RL, Tyler NK, Stovall MY, Chou DL, Putney LF, Gershwin LJ, Schelegle ES, Plopper CG, Hyde DM: Airway generation-specific differences in the spatial distribution of immune cells and cytokines in allergen-challenged rhesus monkeys. *Clin Exp Allergy* 2005;35:894–906.
- 41 Lee YL, Ye YL, Yu CI, Wu YL, Lai YL, Ku PH, Hong RL, Chiang BL: Construction of single-chain interleukin-12 DNA plasmid to treat airway hyperresponsiveness in an animal model of asthma. *Hum Gene Ther* 2001;12:2065–2079.
- 42 Wilson MS, Taylor MD, Balic A, Finney CA, Lamb JR, Maizels RM: Suppression of allergic airway inflammation by helminth-induced regulatory T cells. *J Exp Med* 2005;202:1199–1212.
- 43 Yu CK, Shieh CM, Lei HY: Repeated intratracheal inoculation of house dust mite (*Dermatophagoides farinae*) induces pulmonary eosinophilic inflammation and IgE antibody production in mice. *J Allergy Clin Immunol* 1999;104:228–236.
- 44 Cates EC, Fattouh R, Wattie J, Inman MD, Goncharova S, Coyle AJ, Gutierrez-Ramos JC, Jordana M: Intranasal exposure of mice to house dust mite elicits allergic airway inflammation via a GM-CSF-mediated mechanism. *J Immunol* 2004;173:6384–6392.
- 45 King C, Brennan S, Thompson PJ, Stewart GA: Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium. *J Immunol* 1998;161:3645–3651.
- 46 Gajewska BU, Wiley RE, Jordana M: GM-CSF and dendritic cells in allergic airway inflammation: basic mechanisms and prospects for therapeutic intervention. *Curr Drug Targets* 2003;2:279–292.
- 47 Lewkowich IP, Herman NS, Schleifer KW, Dance MP, Chen BL, Dienger KM, Sproles AA, Shah JS, Kohl J, Belkaid Y, Wills-Karp M: CD4+CD25+ T cells protect against experimentally induced asthma and alter pulmonary dendritic cell phenotype and function. *J Exp Med* 2005;202:1549–1561.
- 48 Huber HL, Koessler KK: The pathology of fatal asthma. *Arch Intern Med* 1922;30:689–760.
- 49 Zhen G, Park SW, Nguyenvu LT, Rodriguez MW, Barbeau R, Paquet AC, Erle DJ: Interleukin-13 and EGF receptor have critical but distinct roles in epithelial cell mucin production. *Am J Respir Cell Mol Biol* 2007;36:244–253.
- 50 Plante S, Semaili A, Joubert P, Bissonnette E, Laviolette M, Hamid Q, Chakir J: Mast cells regulate procollagen I (alpha 1) production by bronchial fibroblasts derived from subjects with asthma through IL-4/IL-4 delta 2 ratio. *J Allergy Clin Immunol* 2006;117:1321–1327.
- 51 Gomes I, Mathur SK, Espenshade BM, Mori Y, Varga J, Ackerman SJ: Eosinophil-fibroblast interactions induce fibroblast IL-6 secretion and extracellular matrix gene expression: implications in fibrogenesis. *J Allergy Clin Immunol* 2005;116:796–804.

- 52 Ramos-Barbon D, Presley JF, Hamid QA, Fixman ED, Martin JG: Antigen-specific CD4(+) T cells drive airway smooth muscle remodeling in experimental asthma. *J Clin Invest* 2005;115: 1580–1589.
- 53 Pegorier S, Wagner LA, Gleich GJ, Pretolani M: Eosinophil-derived cationic proteins activate the synthesis of remodeling factors by airway epithelial cells. *J Immunol* 2006;177:4861–4869.
- 54 Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST: Airway remodeling in asthma: new insights. *J Allergy Clin Immunol* 2003;111:215–226.
- 55 Holgate ST, Holloway J, Wilson S, Bucchieri F, Puddicombe S, Davies DE: Epithelial-mesenchymal communication in the pathogenesis of chronic asthma. *Proc Am Thorac Soc* 2004;1:93–98.
- 56 Temann UA, Geba GP, Rankin JA, Flavell RA: Expression of interleukin 9 in the lungs of transgenic mice causes airway inflammation, mast cell hyperplasia, and bronchial hyperresponsiveness. *J Exp Med* 1998;188:1307–1320.
- 57 Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, Zhang Y, Elias JA: Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eosinophil production. *J Clin Invest* 1999;103:779–788.
- 58 Finotto S, Neurath MF, Glickman JN, Qin S, Lehr HA, Green FH, Ackerman K, Haley K, Galle PR, Szabo SJ, Drazen JM, De Sanctis GT, Glimcher LH: Development of spontaneous airway changes consistent with human asthma in mice lacking t-bet. *Science* 2002;295: 336–338.
- 59 Swirski FK, Sajic D, Robbins CS, Gajewska BU, Jordana M, Stämpfli MR: Chronic exposure to innocuous antigen in sensitized mice leads to suppressed airway eosinophilia that is reversed by granulocyte macrophage colony-stimulating factor. *J Immunol* 2002;169:3499–3506.
- 60 Henderson WR Jr, Tang LO, Chu SJ, Tsao SM, Chiang GK, Jones F, Jonas M, Pae C, Wang H, Chi EY: A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. *Am J Respir Crit Care Med* 2002;165:108–116.
- 61 Tanaka H, Masuda T, Tokuoka S, Komai M, Nagao K, Takahashi Y, Nagai H: The effect of allergen-induced airway inflammation on airway remodeling in a murine model of allergic asthma. *Inflamm Res* 2001;50:616–624.
- 62 Temelkovski J, Hogan SP, Shepherd DP, Foster PS, Kumar RK: An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. *Thorax* 1998;53:849–856.
- 63 Leigh R, Ellis R, Wattie J, Southam DS, De Hoogh M, Gaudie J, O’Byrne PM, Inman MD: Dysfunction and remodeling of the mouse airway persist after resolution of acute allergen-induced airway inflammation. *Am J Respir Cell Mol Biol* 2002;27:526–535.
- 64 Miller LA, Plopper CG, Hyde DM, Gerriets JE, Pieczarka EM, Tyler NK, Evans MJ, Gershwin LJ, Schelegle ES, Van Winkle LS: Immune and airway effects of house dust mite aeroallergen exposures during postnatal development of the infant rhesus monkey. *Clin Exp Allergy* 2003;33: 1686–1694.
- 65 Johnson JR, Wiley RE, Fattouh R, Swirski FK, Gajewska BU, Coyle AJ, Gutierrez-Ramos JC, Ellis R, Inman MD, Jordana M: Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am J Respir Crit Care Med* 2004;169:378–385.
- 66 Angus AC, Ong ST, Chew FT: Sequence tag catalogs of dust mite-expressed genomes: utility in allergen and acarologic studies. *Am J Pharmacogenomics* 2004;4:357–369.
- 67 Krilis S, Baldo BA, Basten A: Antigens and allergens from the common house dust mite *Dermatophagoides pteronyssinus*. II. Identification of the major IgE-binding antigens by crossed radioimmuno-electrophoresis. *J Allergy Clin Immunol* 1984;74:142–146.
- 68 Lind P, Lowenstein H: Identification of allergens in *Dermatophagoides pteronyssinus* mite body extract by crossed radioimmuno-electrophoresis with two different rabbit antibody pools. *Scand J Immunol* 1983;17:263–273.
- 69 Tovey ER, Baldo BA: Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. *J Allergy Clin Immunol* 1987;79:93–102.
- 70 International Union of Immunological Societies – Allergen Nomenclature Sub-Committee, 2006. <http://www.Allergen.Org/>.
- 71 Hewitt CR, Horton H, Jones RM, Pritchard DI: Heterogeneous proteolytic specificity and activity of the house dust mite proteinase allergen Der p 1. *Clin Exp Allergy* 1997;27:201–207.

- 72 King C, Simpson RJ, Moritz RL, Reed GE, Thompson PJ, Stewart GA: The isolation and characterization of a novel collagenolytic serine protease allergen (Der p 9) from the dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1996;98:739–747.
- 73 Stewart GA, Ward LD, Simpson RJ, Thompson PJ: The group III allergen from the house dust mite *Dermatophagoides pteronyssinus* is a trypsin-like enzyme. *Immunology* 1992;75:29–35.
- 74 Yasueda H, Mita H, Akiyama K, Shida T, Ando T, Sugiyama S, Yamakawa H: Allergens from *Dermatophagoides* mites with chymotryptic activity. *Clin Exp Allergy* 1993;23:384–390.
- 75 Wan H, Winton HL, Soeller C, Gruenert DC, Thompson PJ, Cannell MB, Stewart GA, Garrod DR, Robinson C: Quantitative structural and biochemical analyses of tight junction dynamics following exposure of epithelial cells to house dust mite allergen Der p 1. *Clin Exp Allergy* 2000;30:685–698.
- 76 Wan H, Winton HL, Soeller C, Tovey ER, Gruenert DC, Thompson PJ, Stewart GA, Taylor GW, Garrod DR, Cannell MB, Robinson C: Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J Clin Invest* 1999;104:123–133.
- 77 Wan H, Winton HL, Soeller C, Taylor GW, Gruenert DC, Thompson PJ, Cannell MB, Stewart GA, Garrod DR, Robinson C: The transmembrane protein occludin of epithelial tight junctions is a functional target for serine peptidases from faecal pellets of *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2001;31:279–294.
- 78 Chen CL, Lee CT, Liu YC, Wang JY, Lei HY, Yu CK: House dust mite *Dermatophagoides farinae* augments proinflammatory mediator productions and accessory function of alveolar macrophages: implications for allergic sensitization and inflammation. *J Immunol* 2003;170:528–536.
- 79 Sun G, Stacey MA, Schmidt M, Mori L, Mattoli S: Interaction of mite allergens Der p 3 and Der p 9 with protease-activated receptor-2 expressed by lung epithelial cells. *J Immunol* 2001;167:1014–1021.
- 80 Asokanathan N, Graham PT, Stewart DJ, Bakker AJ, Eidne KA, Thompson PJ, Stewart GA: House dust mite allergens induce proinflammatory cytokines from respiratory epithelial cells: the cysteine protease allergen, Der p 1, activates protease-activated receptor (PAR)-2 and inactivates PAR-1. *J Immunol* 2002;169:4572–4578.
- 81 Adam E, Hansen KK, Astudillo OF, Coulon L, Bex F, Duhant X, Jaumotte E, Hollenberg MD, Jacquet A: The house dust mite allergen Der p 1, unlike Der p 3, stimulates the expression of interleukin-8 in human airway epithelial cells via a proteinase-activated receptor-2-independent mechanism. *J Biol Chem* 2006;281:6910–6923.
- 82 Kauffman HF, Tamm M, Timmerman JA, Borger P: House dust mite major allergens Der p 1 and Der p 5 activate human airway-derived epithelial cells by protease-dependent and protease-independent mechanisms. *Clin Mol Allergy* 2006;4:5.
- 83 Hewitt CR, Brown AP, Hart BJ, Pritchard DI: A major house dust mite allergen disrupts the immunoglobulin E network by selectively cleaving CD23: innate protection by antiproteases. *J Exp Med* 1995;182:1537–1544.
- 84 Schulz O, Sutton BJ, Beavil RL, Shi J, Sewell HF, Gould HJ, Laing P, Shakib F: Cleavage of the low-affinity receptor for human IgE (CD23) by a mite cysteine protease: nature of the cleaved fragment in relation to the structure and function of CD23. *Eur J Immunol* 1997;27:584–588.
- 85 Luo HY, Hofstetter H, Banchereau J, Delespesse G: Cross-linking of CD23 antigen by its natural ligand (IgE) or by anti-CD23 antibody prevents B lymphocyte proliferation and differentiation. *J Immunol* 1991;146:2122–2129.
- 86 Yu P, Kosco-Vilbois M, Richards M, Kohler G, Lamers MC: Negative feedback regulation of IgE synthesis by murine CD23. *Nature* 1994;369:753–756.
- 87 Pene J, Chretien I, Rousset F, Briere F, Bonnefoy JY, de Vries JE: Modulation of IL-4-induced human IgE production in vitro by IFN-gamma and IL-5: the role of soluble CD23 (s-CD23). *J Cell Biochem* 1989;39:253–264.
- 88 Sarfati M, Delespesse G: Possible role of human lymphocyte receptor for IgE (CD23) or its soluble fragments in the in vitro synthesis of human IgE. *J Immunol* 1988;141:2195–2199.
- 89 Saxon A, Ke Z, Bahati L, Stevens RH: Soluble CD23 containing B cell supernatants induce IgE from peripheral blood B-lymphocytes and costimulate with interleukin-4 in induction of IgE. *J Allergy Clin Immunol* 1990;86:333–344.

- 90 Machado DC, Horton D, Harrop R, Peachell PT, Helm BA: Potential allergens stimulate the release of mediators of the allergic response from cells of mast cell lineage in the absence of sensitization with antigen-specific IgE. *Eur J Immunol* 1996;26:2972–2980.
- 91 Schulz O, Sewell HF, Shakib F: Proteolytic cleavage of CD25, the alpha subunit of the human T cell interleukin 2 receptor, by Der p 1, a major mite allergen with cysteine protease activity. *J Exp Med* 1998;187:271–275.
- 92 Ghaemmaghami AM, Robins A, Gough L, Sewell HF, Shakib F: Human T cell subset commitment determined by the intrinsic property of antigen: the proteolytic activity of the major mite allergen Der p 1 conditions T cells to produce more IL-4 and less IFN-gamma. *Eur J Immunol* 2001;31:1211–1216.
- 93 Gough L, Schulz O, Sewell HF, Shakib F: The cysteine protease activity of the major dust mite allergen Der p 1 selectively enhances the immunoglobulin E antibody response. *J Exp Med* 1999;190:1897–1902.
- 94 Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY, Cohn L, Hamid Q, Elias JA: Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science* 2004;304:1678–1682.
- 95 Bleau G, Massicotte F, Merlen Y, Boisvert C: Mammalian chitinase-like proteins. *EXS* 1999;87:211–221.
- 96 Boot RG, Blommaert EF, Swart E, Ghauharali-van der Vlugt K, Bijl N, Moe C, Place A, Aerts JM: Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. *J Biol Chem* 2001;276:6770–6778.
- 97 Boot RG, Renkema GH, Verhoek M, Strijland A, Blik J, de Meulemeester TM, Mannens MM, Aerts JM: The human chitotriosidase gene. Nature of inherited enzyme deficiency. *J Biol Chem* 1998;273:25680–25685.
- 98 Elias JA, Homer RJ, Hamid Q, Lee CG: Chitinases and chitinase-like proteins in T(h)2 inflammation and asthma. *J Allergy Clin Immunol* 2005;116:497–500.
- 99 Chang NC, Hung SI, Hwa KY, Kato I, Chen JE, Liu CH, Chang AC: A macrophage protein, ym1, transiently expressed during inflammation is a novel mammalian lectin. *J Biol Chem* 2001;276:17497–17506.
- 100 Ohashi M, Arita H, Hayai N: Identification of a novel eosinophil chemotactic cytokine (ECF-L) as a chitinase family protein. *J Biol Chem* 2000;275:1279–1286.
- 101 McCall C, Hunter S, Stedman K, Weber E, Hillier A, Bozic C, Rivoire B, Olivry T: Characterization and cloning of a major high molecular weight house dust mite allergen (Der f 15) for dogs. *Vet Immunol Immunopathol* 2001;78:231–247.
- 102 Weber E, Hunter S, Stedman K, Dreitz S, Olivry T, Hillier A, McCall C: Identification, characterization, and cloning of a complementary DNA encoding a 60-kd house dust mite allergen (Der f 18) for human beings and dogs. *J Allergy Clin Immunol* 2003;112:79–86.
- 103 Noli C, Bernadina WE, Willemse T: The significance of reactions to purified fractions of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in canine atopic dermatitis. *Vet Immunol Immunopathol* 1996;52:147–157.
- 104 O’Neil SE, Heinrich TK, Hales BJ, Hazell LA, Holt DC, Fischer K, Thomas WR: The chitinase allergens Der p 15 and Der p 18 from *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 2006;36:831–839.
- 105 Aki T, Kodama T, Fujikawa A, Miura K, Shigeta S, Wada T, Jyo T, Murooka Y, Oka S, Ono K: Immunochemical characterization of recombinant and native tropomyosins as a new allergen from the house dust mite, *Dermatophagoides farinae*. *J Allergy Clin Immunol* 1995;96:74–83.
- 106 Tsai LC, Chao PL, Shen HD, Tang RB, Chang TC, Chang ZN, Hung MW, Lee BL, Chua KY: Isolation and characterization of a novel 98-kd *Dermatophagoides farinae* mite allergen. *J Allergy Clin Immunol* 1998;102:295–303.
- 107 Epton MJ, Dilworth RJ, Smith W, Hart BJ, Thomas WR: High-molecular-weight allergens of the house dust mite: an apolipoprotein-like cDNA has sequence identity with the major M-177 allergen and the IgE-binding peptide fragments Mag1 and Mag3. *Int Arch Allergy Immunol* 1999;120:185–191.
- 108 Fujikawa A, Ishimaru N, Seto A, Yamada H, Aki T, Shigeta S, Wada T, Jyo T, Murooka Y, Oka S, Ono K: Cloning and characterization of a new allergen, Mag 3, from the house dust mite,

- Dermatophagoides farinae*: cross-reactivity with high-molecular-weight allergen. *Mol Immunol* 1996;33:311–319.
- 109 Lapa e Silva JR, Possebon da Silva MD, Lefort J, Vargaftig BB: Endotoxins, asthma, and allergic immune responses. *Toxicology* 2000;152:31–35.
 - 110 Reed CE, Milton DK: Endotoxin-stimulated innate immunity: a contributing factor for asthma. *J Allergy Clin Immunol* 2001;108:157–166.
 - 111 Ritz SA, Stämpfli MR, Davies DE, Holgate ST, Jordana M: On the generation of allergic airway diseases: from GM-CSF to Kyoto. *Trends Immunol* 2002;23:396–402.
 - 112 Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K: Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002;196:1645–1651.
 - 113 Tulic MK, Wale JL, Holt PG, Sly PD: Modification of the inflammatory response to allergen challenge after exposure to bacterial lipopolysaccharide. *Am J Respir Cell Mol Biol* 2000;22:604–612.
 - 114 Chisholm D, Libet L, Hayashi T, Horner AA: Airway peptidoglycan and immunostimulatory DNA exposures have divergent effects on the development of airway allergen hypersensitivities. *J Allergy Clin Immunol* 2004;113:448–454.
 - 115 McMillan SJ, Xanthou G, Lloyd CM: Manipulation of allergen-induced airway remodeling by treatment with anti-TGF-beta antibody: effect on the SMAD signaling pathway. *J Immunol* 2005;174:5774–5780.
 - 116 Fattouh R, Midence NG, Arias K, Johnson JR, Lonning S, Gauldie J, Jordana M: Impact of TGF-beta blockade on house dust mite-induced allergic airways inflammation on remodeling (abstract). Meeting of the American Thoracic Society, San Francisco, 2007.
 - 117 Tovey ER, Mitakakis TZ, Sercombe JK, Vanlaar CH, Marks GB: Four methods of sampling for dust mite allergen: differences in 'dust'. *Allergy* 2003;58:790–794.
 - 118 Custis NJ, Woodfolk JA, Vaughan JW, Platts-Mills TA: Quantitative measurement of airborne allergens from dust mites, dogs, and cats using an ion-charging device. *Clin Exp Allergy* 2003;33:986–991.
 - 119 Platts-Mills TA, Vervloet D, Thomas WR, Aalberse RC, Chapman MD: Indoor allergens and asthma: report of the third international workshop. *J Allergy Clin Immunol* 1997;100:S2–S24.
 - 120 Sakaguchi M, Inouye S, Sasaki R, Hashimoto M, Kobayashi C, Yasueda H: Measurement of airborne mite allergen exposure in individual subjects. *J Allergy Clin Immunol* 1996;97:1040–1044.
 - 121 Colloff MJ: Distribution and abundance of dust mites within homes. *Allergy* 1998;53:24–27.
 - 122 Sakaguchi M, Inouye S, Yasueda H, Shida T: Concentration of airborne mite allergens (Der I and Der II) during sleep. *Allergy* 1992;47:55–57.
 - 123 Sakaguchi M, Inouye S, Yasueda H, Irie T, Yoshizawa S, Shida T: Measurement of allergens associated with dust mite allergy. II. Concentrations of airborne mite allergens (Der I and Der II) in the house. *Int Arch Allergy Appl Immunol* 1989;90:190–193.
 - 124 Zock JP, Heinrich J, Jarvis D, Verlato G, Norback D, Plana E, Sunyer J, Chinn S, Olivieri M, Soon A, Villani S, Ponzio M, Dahlman-Hoglund A, Svanes C, Luczynska C: Distribution and determinants of house dust mite allergens in Europe: the European community respiratory health survey II. *J Allergy Clin Immunol* 2006;118:682–690.
 - 125 Lau S, Illi S, Sommerfeld C, Niggemann B, Bergmann R, von Mutius E, Wahn U: Early exposure to house-dust mite and cat allergens and development of childhood asthma: a cohort study. Multicentre Allergy Study Group. *Lancet* 2000;356:1392–1397.
 - 126 Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ: Exposure to house-dust mite allergen (Der p 1) and the development of asthma in childhood. A prospective study. *N Engl J Med* 1990;323:502–507.
 - 127 Owen S, Morganstern M, Hepworth J, Woodcock A: Control of house dust mite antigen in bedding. *Lancet* 1990;335:396–397.
 - 128 Leaderer BP, Belanger K, Triche E, Holford T, Gold DR, Kim Y, Jankun T, Ren P, McSharry, Je JE, Platts-Mills TA, Chapman MD, Bracken MB: Dust mite, cockroach, cat, and dog allergen concentrations in homes of asthmatic children in the northeastern United States: impact of socioeconomic factors and population density. *Environ Health Perspect* 2002;110:419–425.
 - 129 Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, et al: International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483–491.

- 130 Peat JK, Tovey E, Toelle BG, Haby MM, Gray EJ, Mahmic A, Woolcock AJ: House dust mite allergens. A major risk factor for childhood asthma in Australia. *Am J Respir Crit Care Med* 1996;153: 141–146.
- 131 Wickens K, Siebers R, Ellis I, Lewis S, Sawyer G, Tohill S, Stone L, Kent R, Kennedy J, Slater T, Crothall A, Trethowen H, Pearce N, Fitzharris P, Crane J: Determinants of house dust mite allergen in homes in Wellington, New Zealand. *Clin Exp Allergy* 1997;27:1077–1085.
- 132 Bemt L, Vries MP, Knapen L, Jansen M, Goossens M, Muris JW, Schayck CP: Influence of mattress characteristics on house dust mite allergen concentration. *Clin Exp Allergy* 2006;36: 233–237.
- 133 Torrent M, Sunyer J, Munoz L, Cullinan P, Iturriaga MV, Figueroa C, Vall O, Taylor AN, Anto JM: Early-life domestic aeroallergen exposure and IgE sensitization at age 4 years. *J Allergy Clin Immunol* 2006;118:742–748.
- 134 van Strien RT, Koopman LP, Kerkhof M, Spithoven J, de Jongste JC, Gerritsen J, Neijens HJ, Aalberse RC, Smit HA, Brunekreef B: Mite and pet allergen levels in homes of children born to allergic and nonallergic parents: The PIAMA study. *Environ Health Perspect* 2002;110: A693–A698.
- 135 Southam DS, Dolovich M, O’Byrne PM, Inman MD: Distribution of intranasal instillations in mice: effects of volume, time, body position, and anesthesia. *Am J Physiol Lung Cell Mol Physiol* 2002;282:L833–L839.
- 136 Platts-Mills TA, Sporik RB, Wheatley LM, Heymann PW: Is there a dose-response relationship between exposure to indoor allergens and symptoms of asthma? *J Allergy Clin Immunol* 1995;96: 435–440.
- 137 Sporik R, Platts-Mills TA: Allergen exposure and the development of asthma. *Thorax* 2001;56 (suppl 2):ii58–63.
- 138 Upham JW, Holt PG: Environment and development of atopy. *Curr Opin Allergy Clin Immunol* 2005;5:167–172.
- 139 Kuehr J, Frischer T, Meinert R, Barth R, Forster J, Schraub S, Urbanek R, Karmaus W: Mite allergen exposure is a risk for the incidence of specific sensitization. *J Allergy Clin Immunol* 1994;94:44–52.

Manel Jordana, MD, PhD

MDCL 4013, Department of Pathology and Molecular Medicine, McMaster University

1200 Main Street West

Hamilton, ON L8S 3Z5 (Canada)

Tel. +1 905 525 9140, ext. 22473, Fax +1 905 522 6750, E-Mail jordanam@mcmaster.ca