

House Dust Mite Interactions with Airway Epithelium: Role in Allergic Airway Inflammation

Vivek D. Gandhi · Courtney Davidson ·
Muhammad Asaduzzaman · Drew Nahirney ·
Harissios Vliagoftis

© Springer Science+Business Media New York 2013

Abstract House dust mite (HDM) allergens are the most prevalent allergens associated with asthma and rhinitis around the world. The mechanisms of allergic sensitization and allergic airway inflammation after exposure to HDM have been studied extensively, but many questions remain unanswered. Airway epithelial cells are the first line of defense against external antigens and are considered an important player in the development of allergic airway inflammation. Both genetic susceptibility to allergic sensitization and HDM composition play decisive roles in the outcome of HDM-epithelium interactions, especially regarding airway epithelial dysfunction and allergic inflammation. Interactions between HDM and the airway epithelium have consequences for both development of allergy and asthma and development of allergic airway inflammation. This review will describe in detail these interactions and will identify issues that require more study.

V. D. Gandhi · M. Asaduzzaman · D. Nahirney
Pulmonary Research Group, Department of Medicine,
University of Alberta, 567 HMRC,
Edmonton AB T6G 2S2, Canada

V. D. Gandhi
e-mail: vivekdip@ualberta.ca

M. Asaduzzaman
e-mail: masaduzz@ualberta.ca

D. Nahirney
e-mail: nahirney@ualberta.ca

C. Davidson
Pulmonary Research Group, Department of Medicine,
University of Alberta, 559 HMRC,
Edmonton AB T6G 2S2, Canada
e-mail: ced3@ualberta.ca

H. Vliagoftis (✉)
Pulmonary Research Group, Department of Medicine,
University of Alberta, 550A HMRC,
Edmonton AB T6G 2S2, Canada
e-mail: hari@ualberta.ca

Keywords House dust mite · Asthma · Allergy · Allergic sensitization · Inflammatory mediators · Epithelial integrity · Genetic susceptibility · Airway epithelium · Allergic airway inflammation

Introduction

House dust mites (HDM) are one of the most common sources of allergens associated with symptomatic allergic airway diseases in large parts of the world. There are two common species of HDM that are widespread around the world, *D. pteronissinus* and *D. farinae*. More than 20 proteins from HDM have been identified as allergens, including structural proteins and various enzymes. The allergens present in HDM and their properties have been reviewed recently [1]. In addition, these allergens are inhaled together with a variety of bacterial products, including lipopolysaccharide (LPS), which may function as adjuvants for the development of sensitization to HDM allergens.

Recent understanding of the pathogenesis of allergic sensitization and allergic airway inflammation suggests that tissue structural cells play an important role. Specifically for allergic rhinitis and asthma, we know that the airway epithelium is an active participant in disease development. The epithelium forms a barrier between the immune system and the external environment, and dysfunction of the barrier may facilitate allergic sensitization. In addition, the epithelium plays an active role in allergic airway diseases by sensing inhaled allergens and generating signals that activate the immune system.

Interactions of HDM with the Airway Epithelium

HDM has both direct and indirect effects on the airway epithelium. Mediators released following house dust mite-

mediated activation of mast cells or T cells, such as histamine, prostaglandins or IL-13, have multiple effects on epithelial cells. The epithelium therefore may translate and/or propagate HDM-induced signals from immune cells and amplify the inflammatory response without the need for direct interactions with HDM components. However, HDM can also directly activate the airway epithelium and cause many of the changes seen in allergic airway inflammation. In this review we will focus on the direct interactions between HDM and epithelial cells and the role of these interactions in the development of allergic sensitization and allergic airway inflammation.

The main material used to study HDM-epithelial interactions has been HDM aqueous whole body extract, which may be different than the HDM components that we inhale in everyday life. However, there have been no studies to compare the effects of HDM extracts with the effects of house dust containing HDM. Whole-body and fecal material extracts from *D. farinae* contain multiple allergens that may overlap, but the components are not exactly the same [2]. There is also cross reactivity of allergens between species of HDM (*D. pteronissinus* and *D. farinae*), but the antigens again vary to some degree [3]. Studies on the effect of HDM extract on airway epithelial cells have not focused on identifying the functional significance, if any, of these differences. Extracts from different sources vary in their composition; a comparison of extracts from three sources showed that the proteinase content varies significantly and may correlate with the biological effects of these extracts [4].

Airborne products of HDM origin that we inhale in our daily life are complex mixtures of allergens and other structural HDM proteins or proteins of bacterial and fungal origin. The airway epithelium is the first tissue to encounter these antigens. The epithelium expresses many pattern recognition receptors (PRRs) [5] that mediate an innate immune response to HDM. One of the most important contaminants of house dust and HDM extract is LPS, a structural subunit of the bacterial cell wall, which is a TLR4 agonist. There is direct [6] and indirect [7] evidence that HDM-induced airway inflammation is LPS/TLR4 dependent. However, HDM extract also contains allergens that may potentiate the LPS effects on TLR4 [8]. All allergenic extracts contain LPS [9], which in the case of HDM has been attributed to *Bartonella* species in HDM culture medium [10]. Low LPS-containing HDM extracts are now available and have been used for *in vitro* and *in vivo* studies of the effects of HDM on airway epithelium and whole organisms, respectively. However, there have not been good studies comparing the effects of extracts with high and low LPS content. Moreover, some studies have shown that HDM-derived β -glucan acts through TLR2 [11] and dectin-1 [12] to induce immune responses.

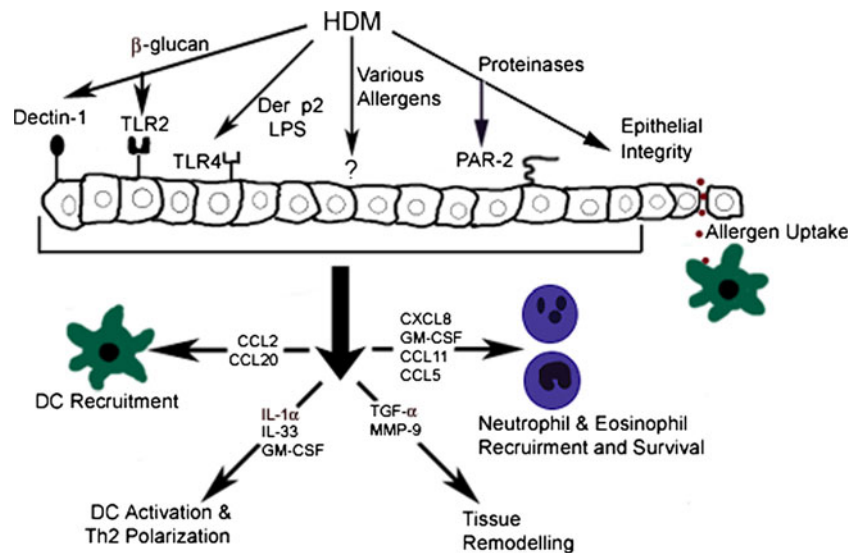
While these PRRs do play a role in sensing allergens, it has recently become clear that the proteinase activity of an allergen can also play an important role in allergic sensitization and inflammation. Serine proteinases may exert their effects through the activation of a receptor expressed on epithelium, called proteinase activated receptor-2 (PAR-2). The role of PAR-2 in airway sensitization and inflammation is described elsewhere in the review. Thus, HDM induces inflammation through various mechanisms on the epithelium, including proteinases, LPS and other TLR agonists (Fig. 1).

Outcomes of HDM-Airway Epithelium Interactions

There is a plethora of studies describing the release of cytokines and chemokines as a result of epithelial cell activation with HDM extracts or with specific HDM allergens. Investigators have used various cell lines or primary cells isolated from different anatomical locations in the respiratory tract for these studies. The available literature, however, is not detailed enough to understand whether HDM has differential effects in proximal airway cells compared to the cells from distal parts of the respiratory tract. For example, certain proteolytic allergens induce eotaxin release from alveolar epithelial cells [13–15], but not from upper airway epithelial cells, although they induce the release of other inflammatory mediators from these cells [16]. Understanding the role of epithelial cells from different anatomical locations in HDM-induced inflammation will be very interesting and biologically important. Airway inflammation is evident throughout the airway tree in asthma [17], but upper airway epithelial cells may be activated by different stimuli than lower airway epithelial cells since only smaller inhaled particles can penetrate deep into the airways and interact with the lower airway epithelial cells.

Differential functional properties of normal and asthmatic epithelial cells are of great interest in understanding the pathogenesis of allergic diseases. It is known that epithelial cells isolated from asthmatics retain functional differences compared to cells from normals after *ex vivo* culture [18]. An important question is whether HDM has differential effects on airway epithelial cells derived from normal and asthmatic tissue. One interesting study showed that only airway epithelial cells from asthmatic individuals released TGF α , a potent epithelial and fibroblast mitogen, upon exposure to HDM or other inflammatory mediators [19]. The same study showed that the cells from asthmatics had a trend toward higher IL-8 and GM-CSF release following stimulation with HDM. Another study showed that primary nasal epithelium derived from HDM-allergic individuals possessed increased basal expression of genes encoding cytokines, chemokines and growth factors compared to cells from non-allergic individuals [20]. The authors speculated

Fig. 1 HDM components and airway epithelial cell receptors that interact to mediate allergic sensitization, airway inflammation and airway remodeling



that this activated profile of the cells from allergic individuals could be responsible for the inflammation seen in these subjects. These differences may be genetic and therefore involved in the manifestation of the asthmatic phenotype, or they may be epigenetic, resulting from chronic airway inflammation or other changes seen in asthma. Whether this effect is the result of different *in vivo* effects of HDM on the epithelium of allergic individuals or indirect effects through HDM-IgE interactions or other interactions was not studied.

Inflammatory mediators released from airway epithelial cells following activation with HDM or specific HDM allergens can have many effects. There is evidence that these mediators may polarize the immune response toward Th2, recruit inflammatory cells including eosinophils and lymphocytes, and also have protective effects.

Role in Allergic Sensitization to HDM

Much of our knowledge on allergic sensitization to HDM comes from animal studies that administer HDM extracts or purified allergens intranasally or by other routes. The overall idea is that HDM induces the release of mediators that can affect dendritic cell (DC) recruitment, antigen uptake, maturation, activation and their subsequent interactions with CD4 T cells and development of Th2 cells.

HDM may recruit DC precursors to the lung through airway epithelium activation. *In vitro* Der p1-mediated activation of airway epithelial cells induces the release of CCL2, CCL20, CCL5, CXCL8 and CXCL10 in a proteinase-dependent fashion [21], and in co-culture systems CCL2 and CCL20 induce recruitment of DC precursors to epithelial monolayers. A requirement for CCL2 in the generation of Th2 immune responses has also been shown in a murine model of ovalbumin-induced allergy [22]. HDM-induced epithelial release of DC chemoattractant CCL20 has been shown to be

mediated by interactions of β -glucan, a fungal moiety possibly from fungal species in the GI tract of HDM [23], with TLR2 [11] or dectin-1 [12] on epithelial cells. The latter effect was mediated by Syk activation and was unique for HDM allergens, since cockroach or pollen allergens did not have the same effect on the airway epithelium. Further, HDM-mediated TLR-4 activation on airway epithelial cells induces IL-1 α , which acts in an autocrine manner to induce the release of the DC chemoattractants GM-CSF and IL-33 [24]. Neutralization of IL-1 α , GM-CSF or IL-33 prevents allergic sensitization to HDM, but TSLP does not seem to have a significant effect in this model. This study proposes that IL-33 is important for OX40L upregulation on DCs [25], which is essential for T cell differentiation to a Th2 phenotype [26]. However, if present in the airways, TSLP could activate recruited DCs to express CD40L and promote Th2 cell development [27]. There is no published evidence that HDM induces TSLP from the airway epithelium, although other allergens, such as *Alternaria*, can do it at least in part through PAR-2 activation [28], a receptor that is also activated by HDM allergens.

Similar studies are difficult to perform in humans. There are a number of human studies using inhalation challenges with HDM extract or isolated HDM allergens that show effects on T and B cell responses [29], eosinophil recruitment [30] or differences between challenges with whole extract versus isolated allergens [31]. However, there is no information regarding the specific role of the epithelium in these effects. The role of the epithelium in human responses to HDM can be extrapolated from the *in vitro* studies with human airway epithelial cells.

Proinflammatory Effects

An important mechanism underlying HDM-induced inflammation is HDM-dependent release of mediators, primarily

cytokines and chemokines, which may induce inflammatory cell recruitment and survival in the airways. Eosinophil infiltration in the airways after allergen exposure [30] is one of the cardinal characteristics of allergic airway inflammation. HDM mediates the release of eotaxin and GM-CSF from airway epithelial cells [13, 32], which may promote eosinophil recruitment and survival, respectively. HDM also induces release of CCL20 [11, 12] and CCL2 [21, 33] from airway epithelial cells, which may act as chemoattractants for immature DCs and monocytes. In addition, HDM showed synergy with IL-4 and TGF β to induce epithelial release of the Th2 chemoattractant TARC [34]. In addition to upregulating chemotactic mediators, HDM upregulates adhesion molecules on epithelial cells [33, 35] and inflammatory cells [35], and this upregulation could facilitate recruitment of inflammatory cells to the airways.

HDM also releases mediators from the airway epithelium whose role in allergic airway inflammation is not as clear, such as IL-6 and IL-8 [32, 33, 36]. There is evidence that IL-8 may be a chemoattractant for eosinophils in allergic individuals [37], but it may also contribute to the neutrophilia seen in the airways in cases of acute asthma exacerbations or severe asthma [38]. An interesting study showed a synergistic effect of HDM and the Th2 cytokines IL-4 and IL-13 in inducing IL-8 and GM-CSF release from airway epithelial cells, indicating that there may be synergism between inflammatory mediators and allergens in perpetuating inflammation [19].

The nervous system is involved in the development of asthma and allergic inflammation. Mouse airway epithelial cells release NGF after Der p 2 activation through ROS-induced p38 and JNK activation [39], and Der p 2 also induces NGF from the airway epithelium *in vivo*. HDM extract also induces NGF release from the epithelium *in vivo*, which is important for the development of characteristics of asthma [40]. Neurotrophin production from the airway epithelium may also prolong eosinophil survival in the airways [41].

Airway remodeling, characterized by goblet cell metaplasia, smooth muscle proliferation and collagen deposition, is another feature of asthma. In addition to airway inflammation, HDM can induce airway remodeling in humans [42] and in animal models [43]. In some cases, these have been attributed to direct effects on the airway epithelium, usually because HDM can have the same effect on airway epithelial cells *in vitro*. For example, HDM promotes epithelial-mesenchymal transition (EMT) in TGF β -activated human airway epithelial cells [44], but not in resting epithelial cells. This observation may explain why the effects of HDM are different in asthma, where there is already airway inflammation, versus normal airway tissue. PAR-2 activation disrupts E-cadherin adhesion between epithelial cells [45], and this may exacerbate remodeling, and it is possible that HDM

mediates the same effects through PAR-2 activation. *In vivo* studies have shown a role for TSLP [46], IL-13 [47] and IL-25 [48] in HDM-induced airway remodeling, but the involvement of direct HDM-epithelium interactions in these effects has not been studied.

In 1998, it was first shown that HDM-induced cytokine release from airway epithelial cells depends on the proteolytic activity of the extracts [32]. It has been shown that these proteinases can activate the airway epithelial receptor PAR-2. PAR-2 activation plays an important role in inflammation [49] by releasing inflammatory mediators [13–16, 50]. In particular, PAR-2 has been implicated in allergic airway inflammation [51, 52]. Activation of PAR-2 plays a role in epithelial barrier dysfunction, which is discussed in the later part of the review. In our laboratory, we have shown that mild inflammation induced by PAR-2 activation in the airways [52] induces allergic sensitization [53] and have data that blocking PAR-2 activation by HDM (unpublished) or cockroach [54] allergens prevents allergic sensitization. An interesting observation is that there is a synergistic effect of PAR-2 activation and LPS on IL-8 release and PAR-2 mRNA expression [55]. PAR-2-mediated release of inflammatory mediators also revealed differential properties of airway epithelial cells present at different locations in the airways. We and others have shown PAR-2-mediated release of eotaxin from small airway epithelial cells [14] and alveolar epithelial cells [13], while upper airway epithelial cells do not release eotaxin upon PAR-2 activation [15, 16].

HDM can also directly activate keratinocytes to release inflammatory mediators [56, 57]. The mechanism of this effect has not been studied in detail, but it may be mediated through PAR-2 activation [58, 59]. Finally, HDM also activates conjunctival cells to release inflammatory mediators [60].

Epithelial Defense Responses to HDM Proteinases

The imbalance between proteinases and antiproteinases in the airways is considered an important factor in the development of allergic airway inflammation [61]. Antiproteinases play an important role in preventing inflammation by neutralizing endogenous or exogenous proteinases, which otherwise could cause tissue destruction. Nasal epithelial cells from subjects with clinical sensitivity to HDM upregulate α 1-antitrypsin upon HDM nasal provocation [62]; these subjects also show increased α 1-antitrypsin levels in nasal lavage compared to subjects without clinical sensitivity to HDM. Primary nasal epithelium of HDM allergic individuals exhibits increased expression of anti-proteinases [20], which may be an attempt by the nasal epithelium to neutralize inhaled allergen proteinases in atopic individuals. At the same time, proteinases in allergens can cleave and inactivate endogenous anti-proteinases. The proteinase activity of Der p 1 can cleave and inactivate α 1-antitrypsin [63] and also decreases the anti-proteinase activity in

BAL fluid [64]. Other anti-proteinases, such as squamous cell carcinoma antigen-2 (SCCA2), are resistant to Der p1-mediated degradation and can irreversibly inhibit catalytic activity of Der p1 and Der f1 mite allergens [65]. These anti-proteinases may function to limit the degree of inflammation and may initiate resolution. IL-4 and IL-13 also induce SCCA1 and SCCA2 release from bronchial epithelial cells [66]. This may be another mechanism to control the inflammatory effects of inhaled allergens.

Other epithelial systems also exhibit similar mechanisms. Cystatin A is released by keratinocytes and can inhibit the proteinase activity of HDM allergens [67]. Physiological levels of SLPI and α 1-antitrypsin inhibit HDM serine proteinase-dependent release of mediators from conjunctival epithelial cells [68].

Other Allergens and the Epithelium – Inflammatory Effects

Other aeroallergens also exert epithelium-dependent effects in allergic inflammation. Fungal proteinases can induce inflammatory mediator release from airway epithelial cells through PAR-2 activation [16, 69] and similar to HDM proteinases can disrupt epithelial integrity [69, 70]. Pollen grains possess NADPH oxidases, which increase ROS in the lung epithelium and recruit neutrophils in the airways [71]. Timothy grass extract and the main allergen Phl p1 induce the release of cytokines from airway epithelial cells [72, 73] but do not affect airway barrier function [72].

The proteinase activity of cockroach allergens has also been studied regarding its role in the development of allergic diseases [74]. There is evidence that CE induces PAR-2-mediated cytokine release from airway epithelial cells [75]. We [54] and others [76] have shown that sensitization to CE and CE-induced airway inflammation is mediated by PAR-2, although there is not good evidence that the latter is dependent on direct epithelial cell activation by cockroach allergens.

Impact of HDM on Epithelial Integrity and Secretory Function

The relationship between exposure to house dust mite allergens and airway barrier function is complex. HDM exposure could be the cause of barrier dysfunction, but at the same time barrier dysfunction may facilitate HDM allergen interactions with immune system components.

Not only are tight junctions (TJ) defective in biopsies from patients with asthma compared to biopsies from control individuals, but also airway epithelial cells cultured from asthmatic individuals show defective tight junction development after *ex vivo* culture [77]. Similarly, the expression of TJ proteins ZO-1 and E-cadherin is decreased in asthmatic airways compared to normal airways [78]. *Ex vivo*

cultured epithelial monolayers show increased permeability and decreased transepithelial resistance, indicating that macromolecules could transverse these monolayers and get access to other tissue components [77].

HDM extracts induce a quick but short-lived decrease in resistance of monolayers of bronchial epithelial cell lines and primary human bronchial epithelial cells [79]. This effect was shown to be dependent on EGF activation. The authors hypothesize that the effect may be PAR-2 dependent, although they do not show any data that direct PAR-2 activation has similar effects. Trypsin on the other hand has been shown to impair epithelial barrier function in a squamous epithelial model through PAR-2 activation on the basolateral surface of epithelial cells [80]. One hypothesis is that HDM, through proteolytic effects or direct effects on TJ proteins, can increase barrier permeability, and then the serine proteinases of HDM can gain access to PAR-2 on the basolateral surface of the airway epithelium and further increase proinflammatory effects. However, the function of HDM allergens could be dependent on circumstances and the composition of the microenvironment. For example, it was shown that EGF can increase barrier function in airway epithelial cells from asthmatic individuals [77]. Serine proteinases can directly transactivate EGF following PAR-2-mediated activation of epithelial cells [81] or can also release EGF ligands [82], so HDM allergens with PAR-2 activating potential may also prevent loss of epithelial barrier integrity. These data indicate that although HDM extracts can alter epithelial permeability *in vitro*, the mechanism of the effect is not clear.

Unfortunately, the HDM extracts used in different studies come from different sources and are not standardized. The effects of HDM extract on epithelial permeability, as well as its effects on cytokine production, depend on the composition of the extract. In one study, the extract with the highest levels of Der p 1 and Der p 2 content but the lowest serine proteinase activity had the largest effect on permeability [4]. The HDM allergen Der p 1 can directly cleave the TJ protein occludin and therefore increase airway epithelial permeability [83]. One study showed that the genes encoding intercellular junction proteins were upregulated in primary nasal epithelial cells of HDM allergic individuals [20], indicating that there may be a constant attempt to restore the epithelium integrity impaired by HDM proteinases.

Another function of the airway epithelium is to secrete fluid that is essential for the proper function of the mucociliary clearance mechanism. In many inflammatory diseases of the airways, including asthma, there is hypersecretion of fluid in the airways. Apical application of HDM extract on normal human nasal epithelial cells increases the ion current, but basolateral application of the extract induces minimal current [84]. This current was dependent on CFTR, CaCC and Ca-activated K channels. The authors also present data that suggest that this effect may be

PAR-2 dependent, although they do not show direct evidence for the role of PAR-2. The data are different from previous reports that showed increased Cl⁻ secretion from epithelial monolayers primarily with basolateral PAR-2 activation [85, 86], while apical activation had no effect [86]. This effect may also be modulated depending on the sensitization status of the organism. We have shown that PAR-2-induced ion currents in whole tracheas are increased in sensitized mice compared to control mice [87]. Apical trypsin and also neutrophil elastase increased Cl⁻ ion currents across monolayers of human nasal and bronchial epithelial cells, but this effect was not mimicked by PAR-2-activating peptides, indicating that it is not PAR-2 dependent [88]. In intestinal epithelial cells, Cl⁻ transport was also activated by basolateral and not apical PAR-2 activation [89].

These results indicate that some of the effects of HDM on epithelial barrier function and ion current may be PAR-2 dependent, but there are also PAR-2-independent components of these effects. Since most of the PAR-2-dependent effects in anion currents are through basolateral PAR-2 activation, increased monolayer permeability following HDM application may also participate in giving access to HDM components to the basolateral surface and therefore influence the PAR-2-dependent effects.

Genetic Variability in the Airway Epithelium and Susceptibility to Asthma

The genetics of allergy and asthma are quite complex. Recent reviews have summarized the knowledge in this field in detail [90–92]. Several studies have linked polymorphisms in epithelially expressed genes to asthma susceptibility. Many of these genes have been confirmed in at least five independent studies, including SPINK5, CC16, CCL5 and CCL11 [91].

In some cases, HDM sensitization has been specifically examined, including the case of CC16 (Clara cell secretory protein). CC16 is secreted by tracheobronchial epithelial cells [93] and is found at lower levels in the BALF of asthmatics than in healthy controls [94]. It appears to act as an antiinflammatory in the lung, possibly decreasing inflammatory cell infiltration [95], and can downregulate Th2 differentiation [96]. Skin prick test positivity and serum IgE levels to HDM were significantly associated with a SNP in the gene that encodes CC16, *SCGB1A1* A38G (rs3741240) [97]. CCL5 and CCL11 are induced by HDM exposure in cultured epithelial cells [13, 98] and therefore are good candidates for HDM-specific asthma susceptibility. Another potentially interesting gene is Cystatin A, a cysteine protease inhibitor, able to inhibit the HDM allergen, Der p 1 [67], for which SNPs have been associated with both atopic dermatitis and increased expression in human lung epithelium [99].

Therefore, polymorphisms in several genes associated with HDM-activated pathways in epithelial cells have been associated with asthma. Determining genetic susceptibility to individual allergens is difficult because of the extremely complex interaction between genetics and the environment. However, the role of these types of genes in HDM-induced asthma is an area that warrants further study and may begin to provide an interesting link between genetic variation in the lung epithelium and HDM sensitivity.

Conclusions

The current literature suggests a very complex network of interactions between HDM and the airway epithelium, which plays a vital role in the generation of allergic inflammation. In many cases, these interactions are similar to the interactions of the epithelium with other allergens and allergenic components. HDM, however, is the most prevalent allergen, and this might indicate that it has some unique ability to interact with the epithelium and cause changes leading to asthma. How these interactions between the epithelium and HDM lead to inflammation and all the cells and cellular mediators that participate in this innate immune response still need to be studied in more detail.

Genetic susceptibility plays an important role in allergic sensitization. Identifying epithelial genetic markers responsible for HDM susceptibility and also for the severity of the allergic disease may allow us to develop improved therapy and may be the first step toward personalized therapy for allergy.

Conflict of Interest Vivek D. Gandhi declares that he has no conflict of interest.

Courtney Davidson declares that she has no conflict of interest.

Muhammad Asaduzzaman declares that he has no conflict of interest.

Drew Nahirney declares that he has no conflict of interest.

Harissios Vliagoftis has received grant support from the CIHR, National Sanitarium Association, Ironwood, Merck & Co., and AstraZeneca, and has received honoraria from Pfizer.

References

1. Gregory LG, Lloyd CM. Orchestrating house dust mite-associated allergy in the lung. *Trends Immunol.* 2011;32(9):402–11.
2. Arlian LG, Bernstein IL, Geis DP, Vyszenski-Moher DL, Gallagher JS, Martin B. Investigations of culture medium-free house dust mites. III. Antigens and allergens of body and fecal extract of *Dermatophagoides farinae*. *J Allergy Clin Immunol.* 1987;79(3):457–66.
3. Arlian LG, Bernstein IL, Vyszenski-Moher DL, Gallagher JS. Investigations of culture medium-free house dust mites. IV. Cross

- antigenicity and allergenicity between the house dust mites, *Dermatophagoides farinae* and *D. pteronyssinus*. *J Allergy Clin Immunol*. 1987;79(3):467–76.
4. Post S, Nawijn MC, Hackett TL, Baranowska M, Gras R, van Oosterhout AJ, et al. The composition of house dust mite is critical for mucosal barrier dysfunction and allergic sensitisation. *Thorax*. 2012;67(6):488–95.
 5. Parker D, Prince A. Innate immunity in the respiratory epithelium. *Am J Respir Cell Mol Biol*. 2011;45(2):189–201.
 6. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med*. 2009;15(4):410–6.
 7. Hongjia L, Qingling G, Meiyang L, Weixuan W, Lihong Z, Yongsheng G, et al. House dust mite regulate the lung inflammation of asthmatic mice through TLR4 pathway in airway epithelial cells. *Cell Biochem Funct*. 2010;28(7):597–603.
 8. Trompette A, Divanovic S, Visintin A, Blanchard C, Hegde RS, Madan R, et al. Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature*. 2009;457(7229):585–8.
 9. Trivedi B, Valerio C, Slater JE. Endotoxin content of standardized allergen vaccines. *J Allergy Clin Immunol*. 2003;111(4):777–83.
 10. Valerio CR, Murray P, Arlian LG, Slater JE. Bacterial 16S ribosomal DNA in house dust mite cultures. *J Allergy Clin Immunol*. 2005;116(6):1296–300.
 11. Ryu JH, Yoo JY, Kim MJ, Hwang SG, Ahn KC, Ryu JC, et al. Distinct TLR-mediated pathways regulate house dust mite-induced allergic disease in the upper and lower airways. *J Allergy Clin Immunol*. 2013;131(2):549–61.
 12. Nathan AT, Peterson EA, Chakir J, Wills-Karp M. Innate immune responses of airway epithelium to house dust mite are mediated through beta-glucan-dependent pathways. *J Allergy Clin Immunol*. 2009;123(3):612–8.
 13. Sun G, Stacey MA, Schmidt M, Mori L, Mattoli S. Interaction of mite allergens Der p3 and Der p9 with protease-activated receptor-2 expressed by lung epithelial cells. *J Immunol*. 2001;167(2):1014–21.
 14. Vliagoftis H, Befus AD, Hollenberg MD, Moqbel R. Airway epithelial cells release eosinophil survival-promoting factors (GM-CSF) after stimulation of proteinase-activated receptor 2. *J Allergy Clin Immunol*. 2001;107(4):679–85.
 15. Rudack C, Steinhoff M, Mooren F, Buddenkotte J, Becker K, von Eiff C, et al. PAR-2 activation regulates IL-8 and GRO-alpha synthesis by NF-kappaB, but not RANTES, IL-6, eotaxin or TARC expression in nasal epithelium. *Clin Exp Allergy*. 2007;37(7):1009–22.
 16. Matsuwaki Y, Wada K, White T, Moriyama H, Kita H. *Alternaria* fungus induces the production of GM-CSF, interleukin-6 and interleukin-8 and calcium signaling in human airway epithelium through protease-activated receptor 2. *Int Arch Allergy Immunol*. 2012;158 Suppl 1:19–29.
 17. van den Berge M, ten Hacken NH, Cohen J, Douma WR, Postma DS. Small airway disease in asthma and COPD: clinical implications. *Chest*. 2011;139(2):412–23.
 18. Gras D, Bourdin A, Vachier I, de Senneville L, Bonnans C, Chanez P. An ex vivo model of severe asthma using reconstituted human bronchial epithelium. *J Allergy Clin Immunol*. 2012;129(5):1259–1266.
 19. Lordan JL, Bucchieri F, Richter A, Konstantinidis A, Holloway JW, Thorner M, et al. Cooperative effects of Th2 cytokines and allergen on normal and asthmatic bronchial epithelial cells. *J Immunol*. 2002;169(1):407–14.
 20. Vrolijk AB, Jonker MJ, Luiten S, Breit TM, Fokkens WJ, van Drunen CM. Primary nasal epithelium exposed to house dust mite extract shows activated expression in allergic individuals. *Am J Respir Cell Mol Biol*. 2008;38(3):293–9.
 21. Pichavant M, Charbonnier AS, Taront S, Brichet A, Wallaert B, Pestel J, et al. Asthmatic bronchial epithelium activated by the proteolytic allergen Der p 1 increases selective dendritic cell recruitment. *J Allergy Clin Immunol*. 2005;115(4):771–8.
 22. Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. *Nature*. 2000;404(6776):407–11.
 23. Bush RK. Indoor allergens, environmental avoidance, and allergic respiratory disease. *Allergy Asthma Proc*. 2008;29(6):575–9.
 24. Willart MA, Deswarte K, Pouliot P, Braun H, Beyaert R, Lambrecht BN, et al. Interleukin-1 controls allergic sensitization to inhaled house dust mite via the epithelial release of GM-CSF and IL-33. *J Exp Med*. 2012;209(8):1505–17.
 25. Chu DK, Llop-Guevara A, Walker TD, Flader K, Goncharova S, Boudreau JE, et al. IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization. *J Allergy Clin Immunol*. 2013;131(1):187–200.
 26. Hoshino A, Tanaka Y, Akiba H, Asakura Y, Mita Y, Sakurai T, et al. Critical role for OX40 ligand in the development of pathogenic Th2 cells in a murine model of asthma. *Eur J Immunol*. 2003;33(4):861–9.
 27. Ito T, Wang YH, Duramad O, Hori T, Delespesse GJ, Watanabe N, et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med*. 2005;202(9):1213–23.
 28. Kouzaki H, O'Grady SM, Lawrence CB, Kita H. Proteases induce production of thymic stromal lymphopoietin by airway epithelial cells through protease-activated receptor-2. *J Immunol*. 2009;183(2):1427–34.
 29. van de Pol MA, Lutter R, van Ree R, van der Zee JS. Increase in allergen-specific IgE and ex vivo Th2 responses after a single bronchial challenge with house dust mite in allergic asthmatics. *Allergy*. 2012;67(1):67–73.
 30. Hatzivlassiou M, Grainge C, Kehagia V, Lau L, Howarth PH. The allergen specificity of the late asthmatic reaction. *Allergy*. 2010;65(3):355–8.
 31. Van Der Veen MJ, Jansen HM, Aalberse RC, van der Zee JS. Der p 1 and Der p 2 induce less severe late asthmatic responses than native *Dermatophagoides pteronyssinus* extract after a similar early asthmatic response. *Clin Exp Allergy*. 2001;31(5):705–14.
 32. King C, Brennan S, Thompson PJ, Stewart GA. Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium. *J Immunol*. 1998;161(7):3645–51.
 33. Osterlund C, Gronlund H, Polovic N, Sundstrom S, Gafvelin G, Bucht A. The non-proteolytic house dust mite allergen Der p 2 induce NF-kappaB and MAPK dependent activation of bronchial epithelial cells. *Clin Exp Allergy*. 2009;39(8):1199–208.
 34. Heijink IH, Marcel Kies P, van Oosterhout AJ, Postma DS, Kauffman HF, Vellenga E. Der p, IL-4, and TGF-beta cooperatively induce EGFR-dependent TARC expression in airway epithelium. *Am J Respir Cell Mol Biol*. 2007;36(3):351–9.
 35. Wong CK, Li ML, Wang CB, Ip WK, Tian YP, Lam CW. House dust mite allergen Der p 1 elevates the release of inflammatory cytokines and expression of adhesion molecules in co-culture of human eosinophils and bronchial epithelial cells. *Int Immunol*. 2006;18(8):1327–35.
 36. Asokanathan N, Graham PT, Stewart DJ, Bakker AJ, Eidne KA, Thompson PJ, et al. 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(8):4572–8.
 37. Sehmi R, Cromwell O, Wardlaw AJ, Moqbel R, Kay AB. Interleukin-8 is a chemo-attractant for eosinophils purified from subjects with a blood eosinophilia but not from normal healthy subjects. *Clin Exp Allergy*. 1993;23(12):1027–36.

38. Ordonez CL, Shaughnessy TE, Matthay MA, Fahy JV. Increased neutrophil numbers and IL-8 levels in airway secretions in acute severe asthma: Clinical and biologic significance. *Am J Respir Crit Care Med*. 2000;161(4 Pt 1):1185–90.
39. Ye YL, Wu HT, Lin CF, Hsieh CY, Wang JY, Liu FH, et al. Dermatophagoides pteronyssinus 2 regulates nerve growth factor release to induce airway inflammation via a reactive oxygen species-dependent pathway. *Am J Physiol Lung Cell Mol Physiol*. 2011;300(2):216–24.
40. Ogawa H, Azuma M, Uehara H, Takahashi T, Nishioka Y, Sone S, et al. Nerve growth factor derived from bronchial epithelium after chronic mite antigen exposure contributes to airway hyperresponsiveness by inducing hyperinnervation, and is inhibited by in vivo siRNA. *Clin Exp Allergy*. 2012;42(3):460–70.
41. Hahn C, Islamian AP, Renz H, Nockher WA. Airway epithelial cells produce neurotrophins and promote the survival of eosinophils during allergic airway inflammation. *J Allergy Clin Immunol*. 2006;117(4):787–94.
42. Phipps S, Benyahia F, Ou TT, Barkans J, Robinson DS, Kay AB. Acute allergen-induced airway remodeling in atopic asthma. *Am J Respir Cell Mol Biol*. 2004;31(6):626–32.
43. Johnson JR, Wiley RE, Fattouh R, Swirski FK, Gajewska BU, Coyle AJ, et al. Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am J Respir Crit Care Med*. 2004;169(3):378–85.
44. Heijink IH, Postma DS, Noordhoek JA, Broekema M, Kapus A. House dust mite-promoted epithelial-to-mesenchymal transition in human bronchial epithelium. *Am J Respir Cell Mol Biol*. 2010;42(1):69–79.
45. Winter MC, Shasby SS, Ries DR, Shasby DM. PAR2 activation interrupts E-cadherin adhesion and compromises the airway epithelial barrier: protective effect of beta-agonists. *Am J Physiol Lung Cell Mol Physiol*. 2006;291(4):628–35.
46. Chen ZG, Zhang TT, Li HT, Chen FH, Zou XL, Ji JZ, et al. Neutralization of TSLP inhibits airway remodeling in a murine model of allergic asthma induced by chronic exposure to house dust mite. *PLoS One*. 2013;8(1):e51268.
47. Tomlinson KL, Davies GC, Sutton DJ, Palframan RT. Neutralisation of interleukin-13 in mice prevents airway pathology caused by chronic exposure to house dust mite. *PLoS ONE [Electronic Resource]* 2010;5(10).
48. Gregory LG, Jones CP, Walker SA, Sawant D, Gowers KH, Campbell GA, et al. IL-25 drives remodelling in allergic airways disease induced by house dust mite. *Thorax*. 2013;68(1):82–90.
49. Bunnett NW. Protease-activated receptors: how proteases signal to cells to cause inflammation and pain. *Semin Thromb Hemost*. 2006;32 Suppl 1:39–48.
50. Vliagoftis H, Schwingshackl A, Milne CD, Duszyk M, Hollenberg MD, Wallace JL, et al. Proteinase-activated receptor-2-mediated matrix metalloproteinase-9 release from airway epithelial cells. *J Allergy Clin Immunol*. 2000;106(3):537–45.
51. Schmidlin F, Amadesi S, Dabbagh K, Lewis DE, Knott P, Bunnett NW, et al. Protease-activated receptor 2 mediates eosinophil infiltration and hyperreactivity in allergic inflammation of the airway. *J Immunol*. 2002;169(9):5315–21.
52. Ebeling C, Forsythe P, Ng J, Gordon JR, Hollenberg M, Vliagoftis H. Proteinase-activated receptor 2 activation in the airways enhances antigen-mediated airway inflammation and airway hyperresponsiveness through different pathways. *J Allergy Clin Immunol*. 2005;115(3):623–30.
53. Ebeling C, Lam T, Gordon JR, Hollenberg MD, Vliagoftis H. Proteinase-activated receptor-2 promotes allergic sensitization to an inhaled antigen through a TNF-mediated pathway. *J Immunol*. 2007;179(5):2910–7.
54. Arizmendi NG, Abel M, Mihara K, Davidson C, Polley D, Nadeem A, et al. Mucosal allergic sensitization to cockroach allergens is dependent on proteinase activity and proteinase-activated receptor-2 activation. *J Immunol*. 2011;186(5):3164–72.
55. Ostrowska E, Sokolova E, Reiser G. PAR-2 activation and LPS synergistically enhance inflammatory signaling in airway epithelial cells by raising PAR expression level and interleukin-8 release. *Am J Physiol Lung Cell Mol Physiol*. 2007;293(5):1208–18.
56. Dai X, Sayama K, Tohyama M, Shirakata Y, Hanakawa Y, Tokumaru S, et al. Mite allergen is a danger signal for the skin via activation of inflammasome in keratinocytes. *J Allergy Clin Immunol*. 2011;127(3):806–14.
57. Kimura T, Sekido M, Chimura N, Shibata S, Kondo N, Kamishina H, et al. Production of GM-CSF mediated by cysteine protease of Der f in canine keratinocytes. *J Vet Med Sci*. 2012;74(8):1033–6.
58. Kato T, Takai T, Fujimura T, Matsuoka H, Ogawa T, Murayama K, et al. Mite serine protease activates protease-activated receptor-2 and induces cytokine release in human keratinocytes. *Allergy*. 2009;64(9):1366–74.
59. Iwakiri K, Ghazizadeh M, Jin E, Fujiwara M, Takemura T, Takezaki S, et al. Human airway trypsin-like protease induces PAR-2-mediated IL-8 release in psoriasis vulgaris. *J Invest Dermatol*. 2004;122(4):937–44.
60. Hara Y, Shoji J, Hori S, Ishimori A, Kato H, Inada N, et al. Evaluation of eosinophilic inflammation in a novel murine atopic keratoconjunctivitis model induced by crude Dermatophagoides farinae antigen. *Allergol intern*. 2012;61(2):331–8.
61. Mautino G, Capony F, Bousquet J, Vignola AM. Balance in asthma between matrix metalloproteinases and their inhibitors. *J Allergy Clin Immunol*. 1999;104(3 Pt 1):530–3.
62. Shin SY, Choi GS, Lee KH, Kim SW, Won KY, Lee JH, et al. Changes of alpha1-antitrypsin levels in allergen-induced nasal inflammation. *Clin Exp Otorhinolaryngol*. 2011;4(1):33–9.
63. Kalsheker NA, Deam S, Chambers L, Sreedharan S, Brocklehurst K, Lomas DA. The house dust mite allergen Der p1 catalytically inactivates alpha 1-antitrypsin by specific reactive centre loop cleavage: a mechanism that promotes airway inflammation and asthma. *Biochem Biophys Res Commun*. 1996;221(1):59–61.
64. Brown A, Farmer K, MacDonald L, Kalsheker N, Pritchard D, Haslett C, et al. House dust mite Der p1 downregulates defenses of the lung by inactivating elastase inhibitors. *Am J Respir Cell Mol Biol*. 2003;29(3 Pt 1):381–9.
65. Sakata Y, Arima K, Takai T, Sakurai W, Masumoto K, Yuyama N, et al. The squamous cell carcinoma antigen 2 inhibits the cysteine proteinase activity of a major mite allergen, Der p 1. *J Biol Chem*. 2004;279(7):5081–7.
66. Yuyama N, Davies DE, Akaiwa M, Matsui K, Hamasaki Y, Suminami Y, et al. Analysis of novel disease-related genes in bronchial asthma. *Cytokine*. 2002;19(6):287–96.
67. Kato T, Takai T, Mitsuishi K, Okumura K, Ogawa H. Cystatin A inhibits IL-8 production by keratinocytes stimulated with Der p 1 and Der f 1: biochemical skin barrier against mite cysteine proteases. *J Allergy Clin Immunol*. 2005;116(1):169–76.
68. Seto T, Takai T, Ebihara N, Matsuoka H, Wang XL, Ishii A, et al. SLPI prevents cytokine release in mite protease-exposed conjunctival epithelial cells. *Biochem Biophys Res Commun*. 2009;379(3):681–5.
69. Kauffman HF, Tomee JF, van de Riet MA, Timmerman AJ, Borger P. Protease-dependent activation of epithelial cells by fungal allergens leads to morphologic changes and cytokine production. *J Allergy Clin Immunol*. 2000;105(6 Pt 1):1185–93.
70. Chen JC, Chuang JG, Su YY, Chiang BL, Lin YS, Chow LP. The protease allergen Pen c 13 induces allergic airway inflammation and changes in epithelial barrier integrity and function in a murine model. *J Biol Chem*. 2011;286(30):26667–79.
71. Boldogh I, Bacsai A, Choudhury BK, Dharajiya N, Alam R, Hazra TK, et al. ROS generated by pollen NADPH oxidase provide a signal that augments antigen-induced allergic airway inflammation. *J Clin Invest*. 2005;115(8):2169–79.

72. Blume C, Swindle EJ, Dennison P, Jayasekera NP, Dudley S, Monk P, et al. Barrier responses of human bronchial epithelial cells to grass pollen exposure. *Eur Respir J* 2012 Nov 8.
73. Roschmann KI, van Kuijen AM, Luiten S, Jonker MJ, Breit TM, Fokkens WJ, et al. Purified Timothy grass pollen major allergen Phl p 1 may contribute to the modulation of allergic responses through a pleiotropic induction of cytokines and chemokines from airway epithelial cells. *Clin Exp Immunol*. 2012;167(3):413–21.
74. Page K. Role of cockroach proteases in allergic disease. *Curr Allergy Asthma Rep*. 2012;12(5):448–55.
75. Hong JH, Lee SI, Kim KE, Yong TS, Seo JT, Sohn MH, et al. German cockroach extract activates protease-activated receptor 2 in human airway epithelial cells. *J Allergy Clin Immunol*. 2004;113(2):315–9.
76. Page K, Ledford JR, Zhou P, Dienger K, Wills-Karp M. Mucosal sensitization to German cockroach involves protease-activated receptor-2. *Respir Res*. 2010;11:62.
77. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, et al. Defective epithelial barrier function in asthma. *J Allergy Clin Immunol*. 2011;128(3):549–56.
78. de Boer WI, Sharma HS, Baelemans SM, Hoogsteden HC, Lambrecht BN, Braunstahl GJ. Altered expression of epithelial junctional proteins in atopic asthma: possible role in inflammation. *Can J Physiol Pharmacol*. 2008;86(3):105–12.
79. Heijink IH, van Oosterhout A, Kapus A. Epidermal growth factor receptor signalling contributes to house dust mite-induced epithelial barrier dysfunction. *Eur Respir J*. 2010;36(5):1016–26.
80. Shan J, Oshima T, Chen X, Fukui H, Watari J, Miwa H. Trypsin impaired epithelial barrier function and induced IL-8 secretion through basolateral PAR-2: a lesson from a stratified squamous epithelial model. *Am J Physiol Gastrointest Liver Physiol*. 2012;303(10):G1105–12.
81. van der Merwe JQ, Hollenberg MD, MacNaughton WK. EGF receptor transactivation and MAP kinase mediate proteinase-activated receptor-2-induced chloride secretion in intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol*. 2008;294(2):G441–51.
82. Darmoul D, Gratio V, Devaud H, Laburthe M. Protease-activated receptor 2 in colon cancer: trypsin-induced MAPK phosphorylation and cell proliferation are mediated by epidermal growth factor receptor transactivation. *J Biol Chem*. 2004;279(20):20927–34.
83. Wan H, Winton HL, Soeller C, Tovey ER, Gruenert DC, Thompson PJ, et al. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *J Clin Invest*. 1999;104(1):123–33.
84. Cho HJ, Choi JY, Yang YM, Hong JH, Kim CH, Gee HY, et al. House dust mite extract activates apical Cl(–) channels through protease-activated receptor 2 in human airway epithelia. *J Cell Biochem*. 2010;109(6):1254–63.
85. Sato S, Ito Y, Kondo M, Ohashi T, Ito S, Nakayama S, et al. Ion transport regulated by protease-activated receptor 2 in human airway Calu-3 epithelia. *Br J Pharmacol*. 2005;146(3):397–407.
86. Palmer ML, Lee SY, Maniak PJ, Carlson D, Fahrenkrug SC, O'Grady SM. Protease-activated receptor regulation of Cl– secretion in Calu-3 cells requires prostaglandin release and CFTR activation. *Am J Physiol Cell Physiol*. 2006;290(4):C1189–98.
87. Rievaj J, Davidson C, Nadeem A, Hollenberg M, Duszyk M, Vliagoftis H. Allergic sensitization enhances anion current responsiveness of murine trachea to PAR-2 activation. *Pflugers Arch*. 2012;463(3):497–509.
88. Swystun V, Chen L, Factor P, Siroky B, Bell PD, Matalon S. Apical trypsin increases ion transport and resistance by a phospholipase C-dependent rise of Ca²⁺. *Am J Physiol Lung Cell Mol Physiol*. 2005;288(5):L820–30.
89. van der Merwe JQ, Moreau F, MacNaughton WK. Protease-activated receptor-2 stimulates intestinal epithelial chloride transport through activation of PLC and selective PKC isoforms. *Am J Physiol Gastrointest Liver Physiol*. 2009;296(6):G1258–66.
90. Meyers DA. Genetics of asthma and allergy: what have we learned? *J Allergy Clin Immunol*. 2010;126(3):439–46. quiz 447–8.
91. Vercelli D. Discovering susceptibility genes for asthma and allergy. *Nat Rev Immunol*. 2008;8(3):169–82.
92. Mukherjee AB, Zhang Z. Allergic asthma: influence of genetic and environmental factors. *J Biol Chem*. 2011;286(38):32883–9.
93. Dierynck I, Bernard A, Roels H, De Ley M. Potent inhibition of both human interferon-gamma production and biologic activity by the Clara cell protein CC16. *Am J Respir Cell Mol Biol*. 1995;12(2):205–10.
94. Van Vyve T, Chanez P, Bernard A, Bousquet J, Godard P, Lauwerijs R, et al. Protein content in bronchoalveolar lavage fluid of patients with asthma and control subjects. *J Allergy Clin Immunol*. 1995;95(1 Pt 1):60–8.
95. Schiffmann E, Geetha V, Pencev D, Warabi H, Mato J, Hirata F, et al. Adherence and regulation of leukotaxis. *Agents Actions Suppl*. 1983;12:106–20.
96. Johansson S, Wennergren G, Aberg N, Rudin A. Clara cell 16-kd protein downregulates T(H)2 differentiation of human naive neonatal T cells. *J Allergy Clin Immunol*. 2007;120(2):308–14.
97. Laing IA, de Klerk NH, Turner SW, Judge PK, Hayden CM, Landau LI, et al. Cross-sectional and longitudinal association of the secretoglobin 1A1 gene A38G polymorphism with asthma phenotype in the Perth Infant Asthma Follow-up cohort. *Clin Exp Allergy*. 2009;39(1):62–71.
98. Stacey MA, Sun G, Vassalli G, Marini M, Bellini A, Mattoli S. The allergen Der p1 induces NF-kappaB activation through interference with IkappaB alpha function in asthmatic bronchial epithelial cells. *Biochem Biophys Res Commun*. 1997;236(2):522–6.
99. Butler MW, Fukui T, Salit J, Shaykhiev R, Mezey JG, Hackett NR, et al. Modulation of cystatin A expression in human airway epithelium related to genotype, smoking, COPD, and lung cancer. *Cancer Res*. 2011;71(7):2572–81.