The Role of the House Dust Mite-Induced Innate Immunity in Development of Allergic Response

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Key Words

Airway epithelial cells • House dust mite • Innate immunity • Lipopolysaccharide

Abstract

House dust mite (HDM) represents one of the most common sources of aeroallergens worldwide and more than 50% of allergic patients are sensitized to these allergenic molecules. HDM allergy research in the past has been mainly focused on adaptive, mite allergen-dependent immune responses. In recent years it has become clear that, although the allergen-specific CD4⁺ Th2 cells orchestrate HDM allergic response, the innate immune system also plays a critical role in HDM-induced allergy pathogenesis. This review will summarize insights into diverse determinants that contribute to the HDM allergenicity through the activation of innate immunity. In addition to the capacity of mite allergens to directly activate mainly skin keratinocytes and airway epithelial cells, innate pattern recognition receptor ligands derived from HDM carriers are also involved in the development of allergic response by HDM.

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Introduction

House dust mite (HDM) sensitization affects more than 15–20% of the population of industrialized countries [1]. A large amount of studies have highlighted the critical role of mite allergen exposures in allergic diseases such as atopic asthma and perennial rhinitis. Moreover, it also appears that mite allergens may represent exacerbating factors for atopic dermatitis (AD) [2].

Analysis of the cellular infiltrate in upper airways, bronchial biopsies and bronchoalveolar lavages, together with the histopathology of AD skin lesions, reveal that allergic rhinitis, atopic asthma and dermatitis share common immunological mechanisms. Furthermore, accumulating reported data demonstrated connections between upper and lower airway inflammation, usually referred to as ‘united airways’ [3]. All these three forms of allergic inflammation are characterized by intense cell infiltrates with degranulated mast cells, eosinophils, dendritic cells (DCs) and allergen-specific Th2 lymphocytes producing cytokines such as interleukin (IL)-4, IL-5, IL-9 and IL-13. Marked epidermal hyperplasia is commonly observed in AD, whereas airway allergy also leads to airway hyperreactivity in both the nose and the lungs as well

as to histological changes of the epithelium and thickening of the basal membrane.

It is increasingly considered that the allergic inflammation results not only from an exacerbated Th2-biased adaptive immune response but is heavily influenced by the direct activation of the innate immune cells such as bronchial epithelial cells, keratinocytes, DCs, mast cells, basophils and eosinophils by both the allergens themselves and danger signals present in the allergen sources.

In addition to their characterized allergens [4], HDMs produce thousands of proteins and macromolecules that might serve for the stimulation of the innate immunity. Whereas many studies evidenced that the biological functions of HDM allergens amplify their allergenicity (see below), new reports demonstrated that contaminating microbial compounds in HDM play a critical role as adjuvant factors to mount a typical Th2-biased allergic responses.

The purpose of this review is to put in evidence the direct activation of innate immune cells by some HDM allergens as well as by contaminating microbial compounds in HDM. We will discuss how the innate immunity triggered by HDM contributes to allergic disease by programming and maintaining Th2-bias adaptive immunity and by the recruitment of inflammatory cells.

Microbial Compounds in HDM Extracts: Biocontaminants or Endosymbiont-Derived Molecules?

The microbial compounds lipopolysaccharide (LPS) and β -glucan can be routinely detected in HDM extracts obtained from whole-mite cultures or mite bodies [5]. Moreover, chitin, a glucosamine-based polymer forming the mite exoskeleton as well as the fungi cell wall, could be more likely present in extracts. Whether these microbial compounds derived from endosymbionts and/or stable contaminants in mite cultures remains to be fully addressed. However, although the presence of large amounts of LPS and/or bacteria as well as β -glucans and/or fungi in house dust, the mites' natural home environment, supports the microbial contamination hypothesis, bacterial 16S ribosomal DNA were identified in washed *Dermaphagoides farinae* or *D. pteronyssinus* as well as in sterilized poultry red mite [6, 7]. These data suggest that endosymbiotic bacteria could also represent the source of contaminating LPS. Ecological relationships between xerophilic fungi and HDM were demonstrated with the presence of penicilloides conidia in the digestive system and fecal pellets of HDM [8]. Moreover, yeast enters fre-

quently in the composition of mite growth medium. Finally, pieces of dust mite cuticle, containing chitin, have been found in fecal pellets [9].

HDM Adjuvant Factors and Airway Innate Immunity

Amongst the microbial compounds contaminating HDM extracts and harboring adjuvant activity, LPS was certainly the most studied danger signal in the context of the airway allergic inflammation.

Animal models provided mechanistic insight into the role of LPS in the regulation of allergic asthma. In relation with the hygiene hypothesis, it appeared that the LPS dose is a determining factor in the course of allergic responses: low doses of inhaled LPS promoted Th2 responses to the sensitizing antigen and eosinophilic inflammation, whereas high doses of LPS induced protective Th1 responses [10].

Using a murine model of HDM allergic asthma (intranasal sensitizations with HDM extracts followed by airway challenge), it was demonstrated that mice deficient in MyD88 or TLR4 did not develop the common features of allergic asthma as airway inflammation, Th2 cytokine production and airway hyperreactivity [11]. This prevention of the allergen-specific Th2 response was associated with fewer OX40L-expressing myeloid DCs in the draining lymph nodes during allergic sensitization. HDM-specific IL-17 production and airway neutrophilia was attenuated in MyD88^{-/-} but not TLR4^{-/-} mice. These data suggested that the presence of microbial products in HDM extracts, more likely LPS, differentially regulates Th2- and Th17-mediated inflammation and activates distinct MyD88-dependent pattern recognition receptors. Whereas the contribution of TLR4 in HDM allergy was clearly evidenced in this report, the nature of the TLR4-positive cells playing a major role in this process remained to be identified.

Very recently, Hammad et al. [12] have investigated the contribution of airway epithelial cells through TLR4 to allergic responses in a murine model of HDM allergy. Using mice with selective ablation of TLR4 expression on either lung structural cells or hematopoietic cells that were treated with HDM extracts, containing 1 ng LPS per mg extract, these authors showed that TLR4 expression on lung structural cells, but not on DCs, is necessary and sufficient for DC activation in the lung and for the development of a robust eosinophilic and Th2 inflammatory response characterized by IL-5 and IL-13 production. TLR4 triggering on structural cells by con-

taminating LPS caused production of the innate pro-Th2 cytokines thymic stromal lymphopoietin (TSLP), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-25 and IL-33. Hammad et al. [12] also established that the absence of Toll-like receptor (TLR) 4 on structural cells, but not on hematopoietic cells, abolished HDM-driven allergic airway inflammation. Finally, inhalation of a TLR4 antagonist to target exposed epithelial cells suppressed the features of asthma, including bronchial hyperreactivity. These new findings give epithelial cells a pivotal position in the generation of allergic inflammation through the activation of TLR4 signaling pathway by the contaminating LPS from HDM.

Intriguing results from in vitro experiments demonstrated that differentiated primary cultures of human airway epithelia or airway epithelial cell lines under a resting state are nevertheless hyporesponsive to LPS in comparison with phagocytic cells [13, 14]. This hyporesponsiveness could be explained by the intracellular TLR4 expression in pulmonary epithelial cells lines [13]. But, more interestingly, a deficiency of MD-2 expression, the LPS-binding accessory TLR4 coreceptor, was observed in the differentiated primary culture of epithelia and extracellular complementation with recombinant MD-2/LPS increased endotoxin responsiveness [14].

Chitin was shown to induce in mice the accumulation in tissue of IL-4-expressing innate immune cells, including eosinophils and basophils [15]. Moreover, chitin induced a dose-dependent expression of AMCse and eotaxin-3 mRNA, two pro-Th2 effector proteins in human sinonasal epithelial cells from patients suffering from chronic rhinosinusitis with nasal polyps [16].

The glucose-derived polymer β -glucan within the HDM extracts was also newly shown to participate in the early events of allergic airway responses. Indeed, HDM extracts induced CCL20 secretion in airway epithelial cells for the recruitment of immature DCs to the lung [17]. The CCL20 production was induced through a TLR-independent, protease-independent process but was dependent on β -glucan structures in the HDM extracts. These effects could be more likely mediated by ligation of HDM-derived β -glucans to non-Toll pattern recognition receptors such as the C-type lectin receptor dectin, although the β -glucan receptor dectin-1 expression was not demonstrated in airway epithelial cells [18]. Whether the β -glucans from HDM, as previously demonstrated for purified fungal β -glucans, activate DCs and eosinophils and aggravate IgE-mediated histamine release remains to be explored [19–21].

Concomitantly, it was newly reported that HDM extracts stimulate cysteinyl leukotriene production by DC through recognition of dectin-2 by glycan-derived molecules [22].

Effects of HDM Extracts on Airway Innate Immune Cells

In this section, we will review studies describing the biological activities of HDM extracts on innate immune cells (table 1), but the precise contribution of the mite allergens or the contaminating microbial compounds in these effects have not been extensively investigated. Notably, the LPS content of HDM extracts has not been systematically specified.

HDM extracts were shown not only to induce increases in the permeability of bronchial mucosa but also to trigger the release of pro-inflammatory cytokines such as IL-6, IL-8, monocyte chemotactic protein (MCP)-1, GM-CSF and IL-1 β in activated airway epithelial cells [23–27]. Both events were largely dependent on protease activity of HDM extracts. These cytokine releases were synergistically increased by cigarette smoke exposure [24, 28]. Interestingly, although similar pro-inflammatory cytokine upregulation was observed in primary bronchial epithelial cultures from healthy individuals or HDM-allergic patients [27], the cytokine secretion by activated bronchial epithelium from allergic patients was synergistically increased in the presence of IL-4 or IL-13. The third main effect of HDM extracts on airway epithelium is the induction of airway remodeling through, at least, transforming growth factor (TGF)- α expression [27]. A very recent paper confirmed that, in TGF- β -primed human bronchial epithelium, HDM was able to promote airway remodeling through epithelial-to-mesenchymal transition characterized by E-cadherin internalization, enhanced β -catenin-dependent transcription and down-regulated cytokeratin [29]. Protease-activated receptor (PAR)-2 activation by HDM extracts led to apical Cl⁻ secretion via cystic fibrosis transmembrane conductance regulator and Ca²⁺-activated Cl⁻ channel and fluid secretion in porcine airway mucosa, suggesting that the protease activity of HDM plays a role in fluid hypersecretion of airway mucosa [30].

Alveolar macrophages stimulated with HDM extracts secreted inflammatory molecules such as nitric oxide, IL-1 β , IL-6, RANTES and tumor necrosis factor (TNF)- α [31, 32]. Interestingly, the pro-inflammatory effect of HDM extract in alveolar macrophage was suggested to be

Table 1. Effects of HDM extracts on airway immune cells

Source	Cell type	Effect	Ref.
Non commercial	Canine MDCK Canine tracheal epithelium Bovine airway epithelium	Cell detachment Increase in bronchial mucosa permeability	23
Non commercial (LPS <0.1 EU/ml)	Human primary nasal epithelium Porcine primary airway mucosa	apical Cl ⁻ channels stimulation through PAR-2 activation	30
ALK	Human A549 Human primary bronchial epithelium	IL-6, IL-8, MCP-1 secretion IL-8, IL-1 β , TGF- α , sICAM-1 and GM-CSF release	25 27, 28
Greer Lab	Human nasal polyp epithelial Human 16HBE14o-, human primary bronchial epithelium Human peripheral blood eosinophil Murine BM-DC	Induction of IL-8, GM-CSF, ICAM-1 expression CCL-20 secretion, airway remodeling (EMT transition) IL-9 release c-KIT, SCF upregulation, IL-6 and cysteinyl leukotriene secretion	26 17, 29 34 22, 36
Allergon	Rat NR8383 alveolar macrophage Murine alveolar macrophage from BAL	Nitric oxide release IL-1 β , IL-6, RANTES and TNF- α release	31 32
Torii Pharma	Human peripheral blood eosinophil	GM-CSF, TNF- α , and IL-8 release	33
CSL	Human MD-DC	Thrombomodulin (CD141) expression	35

MDCK = Madin Darby canine kidney epithelial cells; A549 and 16HBE14o- = human airway epithelial cells; NR8383 = rat alveolar macrophage cells.

associated with binding to AM β 2 integrins and C-type lectins. HDM extracts can activate human eosinophil leading to the production of GM-CSF, TNF- α , IL-8 and IL-9 [33, 34]. It must be pointed out that IL-9 secretion was fully abolished by a serine proteinase inhibitor pretreatment, suggesting a role of PAR-2 in the eosinophil activation by HDM. Finally, HDM extracts upregulate the thrombomodulin (CD141) expression in stimulated DCs and co-cultures of autologous peripheral blood mononuclear cells with CD141-positive DCs enhance Th2-type cytokine production [35]. Control LPS was unable to induce the thrombomodulin expression in DCs. As thrombomodulin is the natural thrombin receptor initiating the anticoagulation pathway, it can be speculated that mite serine proteases are the inducers of thrombomodulin expression in HDM. It is noteworthy that HDM extracts also promoted expression of cell surface c-KIT and its ligand, stem cell factor, on mouse DCs, resulting in sustained signaling downstream of KIT, up-regulation of the Notch ligand Jagged-2 and finally IL-6 secretion [36].

Biological Activity of Purified HDM Allergens and Airway Innate Immunity

Group 1

Whereas it is well known that enzymatically active natural Der p 1 can directly activate the adaptive immune system [37, 38] and, in this way, promotes Th2 sensitization through, at least, the cleavage of CD23 from B cells to upregulate IgE production and CD25 on T cells to downregulate the Th1 cytokine interferon (IFN)- γ [39], group 1 mite allergens also directly stimulate the innate immune system to shape a pro-Th2 environment (table 2).

Specific CD40 and DC-SIGN cleavages in DCs by Der p 1 impaired the Th1 differentiation through the reduction of IL-12p70 and extracellular thiol production as well as a decline in binding to the naïve T cell DC-SIGN ligand intercellular adhesion molecule (ICAM)-3 [40–42].

The proteolytic activity of Der p 1 was recently shown to affect tolerance through downregulation of indoleamine 2,3-dioxygenase expression in DCs from HDM-sensitive subjects with asthma [43]. Upregulation of

Table 2. Effects of group 1 mite allergens on airway innate immunity

Antigen	Cell type/protein	Effect	Ref.
Natural	Human α -antitrypsin, elafin, secretory leukocyte protease inhibitor	Airway antiprotease degradation	44
Natural	Human MD-DC	Suppression of IL-12p70 through CD40 cleavage DC-SIGN cleavage Modulation of IDO expression Cell activation and TARC and MDC upregulation	40 42 43 53, 54
Natural (LPS <0.06 ng/ml)	Canine MDCK Human 16HBE14o-	Increase of epithelium permeability through occluding and ZO-1 cleavages	45
Natural	Human primary bronchial epithelium, Human BEAS-2B, A549	IL-8, IL-6 and GM-CSF, CCL2, CCL5, CCL20 and IP-10 release	47–51
Natural	Human TGF- β latency-associated peptide	TGF- β activation	55
Natural and recombinant (LPS <0.1 EU/ml)	Human blood eosinophil	IL-6, IL-10, TNF- α , GM-CSF and IL-1 β production, CD18 and ICAM-1 upregulation	56, 57
Natural	Human blood basophil Human KU812	IL-4, IL-5, IL-13 upregulation	58
Natural	Rabbit primary airway smooth muscle	Increase in airway smooth muscle responsiveness	59

MDCK = Madin Darby canine kidney epithelial cells; A549, BEAS-2B and 16HBE14o- = human airway epithelial cells; KU812 = human basophilic cells.

indoleamine 2,3-dioxygenase expression was observed in Der p 1-treated DCs from nonatopic subjects. Der p 1-treated DCs from HDM-sensitive patients with asthma co-cultured with autologous CD4⁺ T cells markedly up-regulated GATA-3 and simultaneously downregulated T-bet, leading to an increase in IL-4 production in parallel with a decreasing IFN- γ secretion

Der p 1 degrades airway antiprotease-based lung defenses such as α 1-antitrypsin inhibitor, elafin or secretory leukocyte protease inhibitor [for review, see 44] in mucosa leading to enhanced tissue damage. In addition, Der p 1 and Der f 1 can inactivate lung surfactant proteins A and D, which are known to inhibit the binding of inhaled allergens to cell-sequestered IgE [44]. Der p 1 can digest intercellular tight-junction proteins as occludin and ZO-1 in airway epithelium [45], which increased the permeability of the bronchial epithelium [23, 46] and, consequently, facilitate allergen uptake by DC in subepithelial tissues. Moreover, Der p 1 can directly activate airway epithelial cell lines or bronchial epithelium from patients with allergic asthma to promote pro-inflammatory mediator production such as GM-CSF, IL-6 and IL-8 [47–51], and to increase selective DC recruit-

ment through production of CCL2, CCL20 and IP-10 [54]. Although one previous study demonstrated that this cytokine release is mediated by the PAR-2 activation [51], recent data evidenced, by contrast, that Der p 1 inefficiently cleaves PAR-2 to yield potency activator of the receptor [49, 52] and highlighted that Der p 1-induced cytokine release in airway epithelial cells is more likely independent on PAR-2 activation. The proteolytic activity of Der p 1 was able to activate human myeloid as well as plasmacytoid DCs to initiate Th2 cell responses through costimulatory molecule, HLA-DR expression and pro-Th2 chemokine production as TARC or MDC [53, 54].

Der f 1 can cleave latency-associated peptide of TGF- β and, consequently, induce the activation of latent TGF- β which drives airway remodeling [55]. Group 1 mite allergens (natural or recombinant) were able to induce the direct degranulation of eosinophils but also their activation through pro-inflammatory cytokine (IL-6, IL-10, TNF- α , GM-CSF and IL-1 β) secretion as well as adhesion molecule upregulation (CD18 and ICAM-1) for chemotaxis and transendothelial migration of these cells [56, 57].

In a human basophilic-like cell line (KU812 cells) or in purified peripheral blood basophils, Der p 1 directly activate an IgE-independent but cysteine protease activity-dependent triggering of Th2 cytokines such as IL-4, IL-5 or IL-13 [58]. Finally, proteolytic active Der p 1 can directly elicit changes in airway smooth muscle responsiveness [59].

Group 2

The allergenicity of group 2 mite allergens remained unexplained until recently. However, an elegant study clearly evidenced that Der p 2, by structural homology, acts as a functional homolog of MD-2 to drive airway inflammation in a TLR4-dependent manner [60]. Airway sensitization and challenge with recombinant Der p 2 (0.1 µg), under conditions of very low levels of LPS exposure (0.026 pg), led to experimental allergic asthma in wild-type and MD-2-deficient, but not TLR4-deficient mice. Der p 2 consequently displays auto-adjuvant properties which are critical for the allergenicity of this mite allergen. It is noteworthy that the LPS binding activity of Der f 2 was also recently evidenced [61].

Surprisingly, another study showed that recombinant Der p 2 stimulates airway smooth muscle cells in a TLR4-independent manner but triggered the MyD88 signaling pathway through TLR2 [62].

Lately, natural Der p 2 was shown to elicit the production of TNF-α through direct binding with DC-SIGN on DCs, whereas the unglycosylated recombinant form was ineffective in receptor activation [63]. Recombinant Der p 2 caused nuclear factor κB-dependent upregulation of pro-inflammatory cytokines in bronchial (BEAS-2B and NHBE) but not alveolar (A549) airway epithelial cells [64], whereas recombinant Der f 2 can directly stimulate IL-13 production in BEAS-2B cells [65].

Groups 3, 6 and 9

Der p or f 3, 6 and 9 represent the three HDM serine proteases which display tryptic, chymotryptic and collagenolytic activities, respectively. Similarly to mite cysteine proteases, the serine proteolytic activity of mite proteases was shown to induce an increase in epithelial permeability through cleavage of the tight junction proteins occludin and ZO-1 [66]. Purified natural Der p 3 and Der p 9 induced the release of GM-CSF, eotaxin, IL-6 and IL-8 from cultured airway epithelial cells through PAR-2 activation [47, 49, 67].

Other Mite Allergen Groups

Recombinant Der p 5 stimulated the production of IL-6 and IL-8 in human airway epithelial cells via a protease-independent mechanism [50]. Although to date no studies have evaluated the activation of innate immune cells by group 7 allergens, a very recent report demonstrated that Der p 7 structure displays similarity with that of the LPS-binding protein [68]. Der p 7, contrary to Der p 2 or Der f 2, did not bind LPS but can interact with other lipids including polymyxin B. Consequently, Der p 7 could represent a carrier of bacterial lipopeptides to promote a Th2 response, through putative interactions with TLR2, the receptor of such bacterial ligands. Together with the data obtained with the group 2 and the lipid-binding group 13 and 14 mite allergens [69, 70], lipid-binding properties could represent an important common feature of some HDM allergens. The association of natural lipidic adjuvants with these groups of mite allergens more likely facilitates the allergen sensitization process. The chitinase activity of group 15 and 18 mite allergens [71] could probably amplify the sensitization to HDM as mammalian chitinases or chitinase-like proteins, overproduced under a Th2-bias environment (IL-4, IL-13), may play a key role in the allergic inflammation [72, 73].

AD, Keratinocytes and HDM

HDM allergens were shown to be important factors of AD [74, 75] and the major mite allergens Der p 1 and Der f 1 were detected on the surface of human skin [76]. Proteolytically active HDM allergens not only cause a reduction in the barrier function of the skin [77] but also a delay in epidermal permeability barrier recovery in both human and murine skin [78]. Repeated topical applications of HDM extracts induce severe AD in NC/Nga mice, a sensitive animal model for this human skin disease characterized by inflammatory cell infiltrates in the upper dermis, elevated specific IgE concentrations and CCL20, TARC and eotaxins upregulation [79]. Recent studies indicated that HDM extracts as well as purified natural group 1 mite allergens can directly activate in vitro human and canine epidermal keratinocytes to trigger the secretion of pro-inflammatory cytokines (at least IL-6, IL-8, MCP-1, GM-CSF and TNF-α) [80–82]. Similar GM-CSF and IL-8 production was elicited in mite serine protease-activated keratinocytes through PAR-2 activation [52]. The role of PAR-2 in epidermal permeability

barrier function homeostasis and leukocyte recruitment to inflammatory sites within the skin was clearly evidenced [83]

It must be pointed out that TNF- α is a pivotal pro-inflammatory cytokine for skin inflammation [84] through its role in the expression of TSLP in human keratinocytes. Indeed, TSLP, overproduced in skin keratinocytes during AD, is implicated in the pathogenesis of this disease. Moreover, it was very recently demonstrated that TSLP overexpression by the skin is sufficient to trigger the progression of AD to asthma (atopic march) [85].

Although the role of TLRs in the pathophysiology of AD is not entirely understood, keratinocytes have been shown to express TLRs 1–6 and 9 [86]. Keratinocytes respond to LPS through production of various cytokines and chemokines, including IL-1 β , TNF- α , IL-8, GM-CSF, MIP-1 α and TARC [87–90].

It must be pointed out that HDM extracts stimulated endothelial cells to express ICAM-1, vascular cell adhesion molecule-1 and E-selectin, and to secrete IL-6, IL-8, MCP-1 and GM-CSF through mainly endotoxin contamination [91]. β -Glucans promoted the production of IL-1 β and IL-8 in normal human epidermal keratinocytes [92].

Importance of the Allergen Dose on the Relevance of HDM-Induced Innate Immunity

Although the above-cited results suggest a direct activation of innate immunity by HDM extracts or purified HDM allergens *in vitro* and *in vivo*, the relevance of some of these effects could be considered as questionable as the allergen/microbial compound doses used for cell activation may be much higher from those found in a normal environment [93]. However, any subject is continually in contact with house dust, containing Der p 1 concentrations ranging from 100 ng to 100 μ g/g of fine dust. Consequently, it can be hypothesized that prolonged exposure results in cumulative doses (up to several micrograms per week) of HDM allergens as well as microbial compounds. Moreover, it was demonstrated that HDM fecal pellets (1 fecal pellet contains approx. 0.2 ng of Der p 1), even with a diameter higher than 10 μ m, can be inhaled deep into the lungs. Once in the airways, it was demonstrated that allergens elute from these particles and very high local allergen concentrations can be reached in some areas [94].

It is also interesting to note that mites, eggs or larval forms were detected in human lungs, supporting the high local HDM allergen concentration hypothesis in the airways [95]

HDM-Induced Innate Immunity: Differences between Allergic and Nonallergic Individuals?

Although innate immunity triggered by HDM can influence the Th2-biased allergic response, it is evident that nonallergic subjects, exposed to similar high levels of HDM, can also develop innate immune responses but apparently without consequence on the development of the allergic inflammation. The explanations to this absence of sensitization probably lie in a mixture of genetic susceptibility (possibly through changes in skin and airway structural cells) and the intensity of the innate immunity activation.

Studies with primary cultures of airway epithelial cells from atopic patients have indeed established that the airway epithelium is abnormal. Particularly, it was demonstrated that, at steady state, increased numbers of DCs are observed in the airway mucosa of patients with allergic asthma compared with nonatopic donors, and the density of this network is increased after allergen exposure [96]. Bronchial epithelial cells from allergic asthmatics display an increase in the baseline and Der p 1-induced expression of pro-inflammatory cytokines/chemokines such as GM-CSF, IL-6, IL-8, TSLP, TARC and IP-10 compared with nonatopics [51, 97].

Whereas normal skin and airway epithelium were reported to express undetectable and low-amount TSLP, respectively, TSLP was overexpressed at the lesional sites of AD and in the bronchial epithelium and submucosa in asthmatics [97, 98]. Moreover, PAR-2 overexpression together with a prolonged and robust airway epithelial NF- κ B activation in bronchial biopsies from allergic patients compared with nonatopics are indications that HDM-induced innate immunity in sensitized patients can be exacerbated, which reach a threshold to trigger and/or sustain the allergic response [99, 100]. Moreover, it must be pointed out that the frequency of Tregs, which can negatively regulate the pro-Th2 effects of innate immunity, is reduced in atopics [101, 102], whereas increased infiltration of CD4⁺/CCR4⁺ T cells is detected in the asthmatic bronchial biopsies [97].

Concluding Remarks

Rapid progress in molecular biology of HDM allergens has advanced our knowledge on the structure/function relationships of these antigens. Recent studies put in evidence that, roughly, at least two classes of HDM allergens drastically amplify the allergic response through modulation of the innate immunity: the group 1, 3, 6 and 9 proteases through direct cell activation and the group 2, 7 and probably 13 and 14 lipid-binding proteins through facilitated transport of microbial lipid compound adjuvants. Consequently, even HDM allergens with low IgE-binding activity (at least groups 3, 6, 9, 13 and 14) could activate innate immune cells to initiate/prolong the HDM-induced allergy pathogenesis. Some recent studies brought new insights into the critical role of contaminating LPS and β -glucans which appeared to be at least as important as the intrinsic allergenicity of

HDM allergens to control the Th2 polarization. Whether the presence of these microbial compounds results from endosymbiosis or stable contaminations remains to be investigated, but the modulation of the HDM micro-environment could drastically influence HDM allergenicity.

Future research should try, using purified natural or recombinant mite allergens, to elucidate whether exposure to these individual mite allergens, even displaying low IgE-binding activity, are able to stimulate innate immunity through the production of the pro-Th2 cytokines as TSLP to skew the immune response to Th2. Extensive studies should also focus on the putative additional adjuvant molecules from HDM which could influence the outcome of the responses to HDM allergens. The characterization of HDM allergenicity could thus open the door to new therapeutic approaches.

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