New Insights into the Molecular Basis of the House Dust Mite-Induced Allergic Response

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Abstract: House dust mite (HDM) represents world-wide one of the most common source of aeroallergens word-wide and more than 50% of allergic patients are sensitized to these allergenic molecules. Although the induction of specificTh2 cells as well as IgE by HDM is well understood, the events that control the initial Th2 polarization in response to HDM are still poorly defined. Notably, mechanisms by which HDM is recognized by the airway mucosa, interacts with barrier epithelial cells, leading to dendritic cell (DC) recruitment, activation, and subsequent Th2-mediated responses, remains to be elucidated. Moreover, whereas the allergenicity of the group 1 major mite allergens could be largely explained by their intrinsic proteolytic activity, the fundamental mechanistic question regarding the putative intrinsic allergenic properties of the group 2 major mite allergen remained unanswered to date.

This review summarizes new insights into diverse determinants that contribute to the HDM allergenicity. In addition to the auto-adjuvant capacity of the two major mite allergen Der p 1 and 2, due to proteolytic activity and functional mimicry of the Toll-like receptor 4 (TLR4) co-receptor MD2 respectively, contaminating factors derived from HDM carriers, mainly endotoxins (LPS) et β-glucans, are very important to activate the innate immune response which, in turns, is involved in the development of allergic response by HDM.

INTRODUCTION

Allergic reactions are symptomatic responses to a normally innocuous environmental antigen such as pollen, animal dander and house dust mites. The prevalence of allergic asthma, the most common allergic disease, increased dramatically in the last 50 years, ranging from 5 to 30% in the developed countries [1]. This exacerbated immune-mediated disorder is characterized by chronic airway inflammation, mucus production, and variable airflow obstruction with airways hyperresponsiveness (AHR) [2].

House dust mites can be considered as a predominant provider of inhalant allergens within the world and the mite sensitization affects more than 15-20% of the population from industrialized countries [3]. The most common mite species *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* produce more than 20 different allergen groups classified according to their sequence homology and biological function [4-7]. Despite increasing knowledge about the molecular and functional characteristics of HDM allergens, little is known about the mechanisms that control the initial events of the HDM-induced allergic response.

The purpose of this review is to discuss recently identified functions of contaminating microbial compounds in HDM in the initiation of the allergic response through direct effects on the innate immune system but also through their influence on the group 2 mite allergen allergenicity. This review outlines that, in addition to the intrinsic biological functions of HDM allergens, non-allergenic factors from their carrier contribute to the HDM allergenicity

CRITICAL ROLE OF TH2 CELLS IN ALLERGY AND OF DENDRITIC CELLS IN THE TH2 PRIMING

Following contacts with inhaled antigens and depending on the susceptibility of the individual, either a healthy or an allergic immune response to the antigen arises. The normal pulmonary response to harmless airborne particles is tolerance through the action of allergen-specific Treg cells and the production of IgG4 or IgG1 allergen-specific antibodies [8]. There is no specific production of IgE and no development of airway inflammation following chronic exposure. In contrast, atopic subjects display reduced frequencies of allergen-specific Treg cells [9]. Experimental evidences suggest that allergen-specific Th2 cells play the central role for the primary mediation of the asthmatic inflammatory response [10] as an increase in Th2 lymphocytes was observed in the airways of human asthma patients and in animals sensitized and challenged with allergens [11-18]. The secretion by Th2 cells of IL-4, IL-5, IL-9, and IL-13 [19-21] and probably other recently identified cytokines, such as IL-25, IL-31, and IL-33 mediate the allergen-specific Th2 response and inflammation [22-26] characterized by the production of allergen-specific IgE, eosinophilia, the permissiveness of endothelium for the recruitment of inflammatory cells to

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1874-8384/09

inflamed lungs, the production of mucus, and the modulation of the airway smooth muscle contraction [27].

However, some studies also clearly evidenced that other T cell subsets, including Th1 cells, natural killer T (NKT) cells, Th17 cells, γδT cells and CD8+T cells accumulate in the airway of asthmatics and may also play a role in its pathogenesis [28].

Whereas biology of Th2 cells and their importance for the immediate and chronic phases of the allergic responses are well understood, little is known about the mechanisms that control the initial Th2 polarization in response to allergens.

Many studies report that dendritic cells (DCs), the most powerful antigen presenting cells (APCs), orchestrate the induction of Th1, Th2, Th17 or Treg responses [29, 30]. Many factors are decisive in the process of Th polarization including the type of antigen, the presence of microbial compounds, the route of exposure and the genetic background of the host. All these factors will act at the level of DC to induce signals triggering Th subset cell differentiation [2,31].

Consequently, airway DCs must be critical for priming and Th2 differentiation of naïve T cells towards aeroallergens [32]. A network of airway DCs is located just under the epithelial layer to act as sentinels for invading pathogens and inhaled antigens. DCs can sample the allergens present the airway lumen by forming dendritic extensions between epithelial cells or the allergens gain access to DCs through cleavage of tight junction proteins (see below). Aeroallergens are then captured by these DCs through endocytosis followed by their processing and their presentation to CD4 T-helper (Th) cells [33]. The allergen peptide presentation by MHC class II molecules to naïve CD4+ Th cells requires subsequent migration of DCs that have captured antigen to the T cell area of mediastinal lymph nodes. During this event, DCs acquire a mature phenotype characterized by the up-regulation of the co-stimulatory molecules CD80 and CD86 for naïve T cell activation [34].

However, the allergen peptide presentation to TcR and the interaction of costimulatory molecules from DC with the corresponding receptors in T cells are insufficient to mount T-cell responses. It requires also additional and critical regulatory signals: the presence of DC-activating cytokines produced notably by the airway epithelial cells, the cytokines released from DC as well as adjuvant factors from the microenvironment which influence these cytokine productions. It must also be pointed out that, whereas conventional DCs are important for generating T cell division and priming, plasmacytoid DCs (pDCs) suppress T cell effector generation to promote tolerance. Strikingly, exposure to harmless antigen in the absence of pulmonary pDCs led to Th2 cell-induced allergic asthma [35].

The DC-derived cytokines IL-12 and IL-10 are the key polarizing components to induce Th1 or Treg responses respectively whereas IL-6, IL-21, IL-23, IL-1β are skewing signals for differentiation of Th17 cells [36, 37]. In contrast, a DC-derived cytokine skewing to Th2 was not described to date. Notably, these APCs are not able to produce the key Th2-polarizing cytokine IL-4. However, a recent observation indicated that allergen-activated basophils could be the initial source of Th2-inducing cytokines [38]. Indeed, direct activation of basophils by protease allergens lead to the recruitment of basophils to T-cell areas of draining lymph nodes during the priming phase of the response and to the production of IL-4 and Thymic stromal lymphopoietin (TSLP) which are involved in Th2 differentiation *in vivo*. The results clearly confirm that innate immune cells are major participants in the initiation of allergen-specific Th2 cell responses.

CRITICAL ROLE OF TH2-PROMOTING ADJUVANT SIGNALS IN DC ACTIVATION

Although the sensitization process to natural allergens remains to be fully elucidated, it is evident that, for the priming of the allergic response, DCs need to be activated by a sense of danger triggered by "adjuvant factors" from the microenvironment. These Th2-promoting adjuvant signals can derive from the intrinsic properties of the allergen itself, as clearly demonstrated with Der p 1 [39 and see below], from the allergen carriers as phytoprostanes from pollen grains or endotoxins from HDM [40, 31] or from exposures to environmental components present in air and dust such as respiratory viruses, air pollutants, cigarette smoke or microbial products [41,42]. All these inhaled agents could directly shape a pro-Th2 DC and/or stimulate the activation of the airway epithelium which, in turn, will communicate with DC to induce Th2-biased responses.

Consequently, to induce allergen sensitization, individuals must be not only exposed to the allergen but also to components that directly activate receptors expressed on cells of the innate immune system [43]. For example, the microbial-derived products, the so-called pathogen-associated molecular-pattern (PAMP) molecules act through recognition by pattern-recognition receptors (PRRs), including the Toll-like receptors (TLRs), NOD-like receptors (nucleotide-binding oligomerization domain containing proteins NLRs), C-type lectin receptors including Dectin, and dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN/CD209) [44-46].

THE DOUBLE-EDGED EFFECT OF ENDOTOXIN IN ALLERGIC INFLAMMATION

The bacterial lipopolysaccharide (LPS), the endotoxin of Gram-negative bacteria activating the TLR4 signaling pathway, is one of the most important microbial products which contaminates inhaled allergens as cockroachs, pollen and HDM. Allergen sensitization and development of atopic asthma were shown to be inversely related to endotoxin levels in house dust [47,48]. Endotoxin uptake also depends on the membrane-bound or soluble form of CD14 receptor [49]. Interestingly, polymorphisms of the genes encoding TLR4 and CD14 were found to influence the severity of asthma and its relation with endotoxin exposure [50-52].

However, epidemiological studies indicate that LPS exposure can exacerbate established asthma, probably by stimulating pro-inflammatory responses in the airways [47,53]. Animal models provided mechanistic insights 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 re-

sponses to the sensitizing antigen and eosinophilic inflammation, whereas high doses of LPS induced protective Th1 responses with, however [54] suppressed expression of Th2-type cytokines and decreased eosinophilic inflammation and airway hyperresponsiveness but induced neutrophil inflammation [55]. Interestingly, the pro-Th2 adjuvant effect of low doses of LPS was found to depend on the Myd88-dependent signaling pathway, at least when administered by the airway route [56], by inducing the expression of inflammatory cytokines such as IL-6, IL-12 and TNF-α [54, 56-58].

MEDIATION OF THE ALLERGIC RESPONSE BY AIRWAY EPITHELIAL-CELL–DC INTERACTIONS

The action of TLR4 ligands was particularly well studied at the level of DCs because of their central role in the instruction of Th2 cells, which orchestrate the allergic reaction [59,60]. But in the context of allergic inflammation, epithelial cells also represent potential targets for such microbial products as airway epithelial cells have been shown to express TLR4 and can be activated by LPS. Consequently, although the epithelium was initially considered to function solely as a physical barrier, it is now considered as a central player in the Th2-cell sensitization process by mediating innate immune responses through cross-talk with DCs [32].

In response to allergens, TLR ligands or ambient particulate matter, airway epithelial cells trigger DC migration into epithelium via CCL20 (MIP-3α) production [32] as CCL20 is the only chemokine known to interact with CCR6 that is expressed by immature DC and Langerhans cells. The importance of CCR6 in allergic pulmonary inflammation has recently been demonstrated by using a cockroach antigen model with $CCR6^{-/-}$ mice, showing that lack of CCR6 attenuates the allergic airway response to cockroach antigen [61]. Recent studies have shown that airway epithelial cells also influence DC control of Th differentiation locally via notably the production of the DC activator TSLP, IL-33 or IL-25. TSLP induces the expression of OX40 ligand on dendritic cells that triggers inflammatory Th2 differentiation in the absence of IL-12 through interactions with the T cell OX40 [62]. IL-33 was shown to enhance the production of IL-5 and IL-13 by Th2 cells but not by Th1 cells *in vitro* [63]. IL-25 regulate also adaptive immunity by enhancing Th2 cytokine productions and induced AHR through activation NKT cells expressing IL-17RB [64,65]. A recent report showed that airway epithelial cells can produce IL-25 in response to an innate immune response to allergen [66].

CONTAMINATING LPS IS ESSENTIAL FOR THE INDUCTION OF HDM ALLERGY THROUGH TLR4 SIGNALING

Using a murine model of HDM allergic asthma (intranasal sensitizations with HDM extracts followed by airway challenge), it was lately demonstrated that mice deficient in MyD88 or TLR4 did not develop the common features of allergic asthma as airway inflammation, Th2 cytokine production and airways hyperreactivity [67]. This prevention of the allergen-specific Th2 response was associated with fewer OX40L-expressing myeloid dendritic cells 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 regulate Th2- and Th17-mediated inflammation and activate 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+ cells playing a major role in this process remained to be identified.

Very recently, Hammad *et al.* investigated the contribution of airway epithelial cells through TLR4 to allergic responses [68]. This group first demonstrated that TLR4 was expressed predominantly on pulmonary epithelial cells and alveolar macrophages and that TLR4 expression on airway structural cells was vital for effective migration of dendritic cells. Using mice with selective ablation of TLR4 expression on either lung structural cells or hematopoietic cells and that were treated with HDM extracts, 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 caused production of the innate pro-Th2 cytokines TSLP, GM-CSF, IL-25 and IL-33. The absence of TLR4 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. The 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.

OTHER IMMUNOSTIMULATORY FACTORS CONTRIBUTING TO HDM ALLERGENICITY

Other contaminating products than LPS might regulate the HDM-induced allergic diseases. Chitin, a widespread environmental biopolymer of N-acetyl-β-D-glucosamine, part of the house dust mite exoskeleton, was shown to induce in mice the accumulation in tissue of IL-4-expressing innate immune cells, including eosinophils and basophils [69]. Moreover, Chitin induced a dose-dependent expression of acidic mammalian chitinase (AMCase) and eotaxin-3 mRNA, two pro-Th2 effector proteins in human sinonasal epithelial cells from patients with chronic rhinosinusitis with nasal polyps (CRSwNPs) [70].

The glucose-derived β-glucan polymers within the HDM extract were also newly shown to participate in the early events of allergic airway responses. Indeed, HDM induced CCL20 secretion in airway epithelial cells for the recruitment of immature DCs to the lung [71]. The effect was HDM-specific because other aeroallergens, such as ragweed and cockroach, fail to elicit this response. The CCL20 production was induced through a TLR-independent, protease-independent process but was dependent on β-glucan structures in the HDM extracts as other β-glucans could competitively inhibit the CCL20 secretion and as β-glucanase-treated HDM significantly failed to trigger the subsequent chemokine secretion. These effects could be more likely mediated by ligation of HDM-derived β-glucans to non-Toll PRRs such as the C-type lectin receptor dectin.

Concomitantly, it was newly reported that HDM extracts stimulate cysteinyl leukotrienes (Cys-LTs) production by DC through recognition of Dectin-2 by glycan-derived molecules [72]. These compounds could be isolated by Concanavalin A affinity chromatography, suggesting a mannose- or glucose-rich ligand. Cys-LTs through interactions with type 1 or 2 receptors, are potent mediators of bronchial smooth muscle constriction, vascular permeability, and pulmonary inflammation in bronchial asthma. This leukotriene generation through Dectin-2 activation was also dependent FcRγ /Syk signaling pathway. This new pathway may activate innate immune cells to promote allergic inflammation. However, whether the critical glycan(s) binding to Dectin-2 is one of the many recognized glycoproteins in house dust mite remains to be determined.

It is noteworthy that HDM extracts promoted expression of cell surface c-KIT and its ligand, stem cell factor, on mouse DCs, resulting in sustained signaling downstream of KIT, upregulation of the Notch ligand Jagged-2, and finally IL-6 secretion [73]. The authors hypothesize that IL-6 upregulation could limit the Th1 response while promoting the Th2 and Th17 pathways. It must be pointed out that the role of IL-6 was evidenced in Th2 and Th17 response elicited by allergens [74]. The nature of the compounds within the HDM extracts eliciting the IL-6 up-regulation at the level of DC remains to be identified. Transactivation of c-Kit by Cys-LTs, as already demonstrated in mast cells [75], could be another mechanism by which cys-LTs produced in response to house dust mite can modulate DC function in an autocrine fashion to promote allergic inflammation.

BIOLOGICAL FUNCTIONS OF GROUP 1 AND 2 MITE ALLERGENS

Group 1 and 2 mite allergens represent the most important allergenic molecules among HDM antigens as the vast majority of HDM-allergic patients develop specific IgE to these allergens and as 50–100% of IgE reactivity to HDM extract was directed towards these proteins [76-79]. Due to the wide cross-reactivity among mite species, active immunotherapy against Der p 1 and Der p 2 would potentially cure 80% of all mite-allergic patients globally [76,77]. Consequently, extensive studies for a greater understanding of the allergenicity of group 1 and 2 HDM allergens could help to define new therapeutic strategies to block the allergic reaction induced by these antigens.

It is well known that the proteolytic activity of Der p 1 can directly activate the innate and adaptive immune systems and, in this way, promotes Th2 sensitization [39 for a review]. Indeed, Der p 1 can cleave several cell-surface molecules, such as CD23 from B cells to up-regulate IgE production. Der p 1 can also cleave CD25 on T cells, CD40 and DC-SIGN from DC to polarize the immune system to the Th2 bias. Animal models demonstrated that the Der p 1 proteolytic activity is crucial for allergic sensitization and can even facilitate sensitization to bystander antigens. Der p 1 degrades airway antiproteases, such as α1-antitrypsin inhibitor, elafin, or secretory leukocyte protease inhibitor. 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. Der p 1can degrade tight-junction proteins in airway epithelium, which increased the permeability of the bronchial epithelium and consequently, facilitate allergen uptake by DC in subepithelial tissue. Moreover, Der p 1 can directly activate airway epithelial cells to promote proinflammatory cytokine production such as IL-6, IL-8 in a PAR2-independent manner [80].

Recently, it was demonstrated that Der p 1-induced degradation of CD40 resulted in reduced production of extracellular thiols by DC, and polarizing naive T cells towards Th2 because thiols appeared to be more critical for the generation of Th1 than Th2 cells [81].

The precise mechanism by which the major mite allergen Der p 2 favour Th2 allergic responses remained unresolved until recently. However, an elegant study clearly evidenced that Der p 2, by structural homology, acts as a functional homologue of MD2 (a lipid-binding, Toll-like receptor 4 (TLR4) signalling co-factor) to drive airway inflammation in a TLR4- dependent manner [82]. Indeed, the ability of Der p 2, purified from house dust mites and containing low levels of LPS, to activate cells in the absence of MD2 in a TLR4-specific manner indicated that an LPS–Der p 2 complex might mimic the TLR4-activating properties of the LPS–MD2 complex. Moreover, recombinant Der p 2 devoided of any LPS was inactive in the functional assays. Airway sensitization and challenge with Der p 2 (0,1μg), under conditions of very low levels of LPS exposure (0,026pg) normally would induce tolerance, led to experimental allergic asthma in wild type and MD-2-deficient, but not TLR4-deficient mice. Because Der p 2 mimics the function of MD2, Der p 2 consequently displays auto-adjuvant properties which are critical for the allergenicity of this mite allergen.

It must be pointed that many allergens, including Der p 2, are members of the MD2-like lipid binding protein family [83], suggesting that the intrinsic adjuvant activity provided by associated lipids could also contribute to the allergenicity of these allergens.

Surprisingly, another study showed that Der p 2 stimulates airway smooth muscle cells in a TLR4-independent manner and leading to nuclear factor-kappa B (NF-κB), ERK, JNK activations, c-Fos expression and a high level of proinflammatory cytokines MCP-1, IL-6 and eotaxin expression which are important factors for the initiation of the Th2 allergic response [84]. Der p 2, on these cells, triggered the MyD88 signaling pathway through TLR2.

CONCLUDING REMARKS

HDM represents one of the most allergen carrier within industrialized countries. Although rapid progress in molecular biology of HDM allergens advanced our knowledge on the structure/function relationships of these antigens, several important questions remained unanswered about the HDM allergenicity. Some recent studies brought new insights into the critical role of contaminating LPS and β-glucans to control the initial Th2 polarization in response to HDM allergens. These non-allergenic adjuvant factors cooperate together with at least group 1 and 2 mite allergens, which display their own auto-adjuvant capacity, to stimulate not only DC but also airway epithelial cells to skew the immune response to Th2. On the basis of these findings, we proposed a new model of the innate initiation of the HDM-induced allergic response as illustrated in detail in Fig. (**1**). Future re-

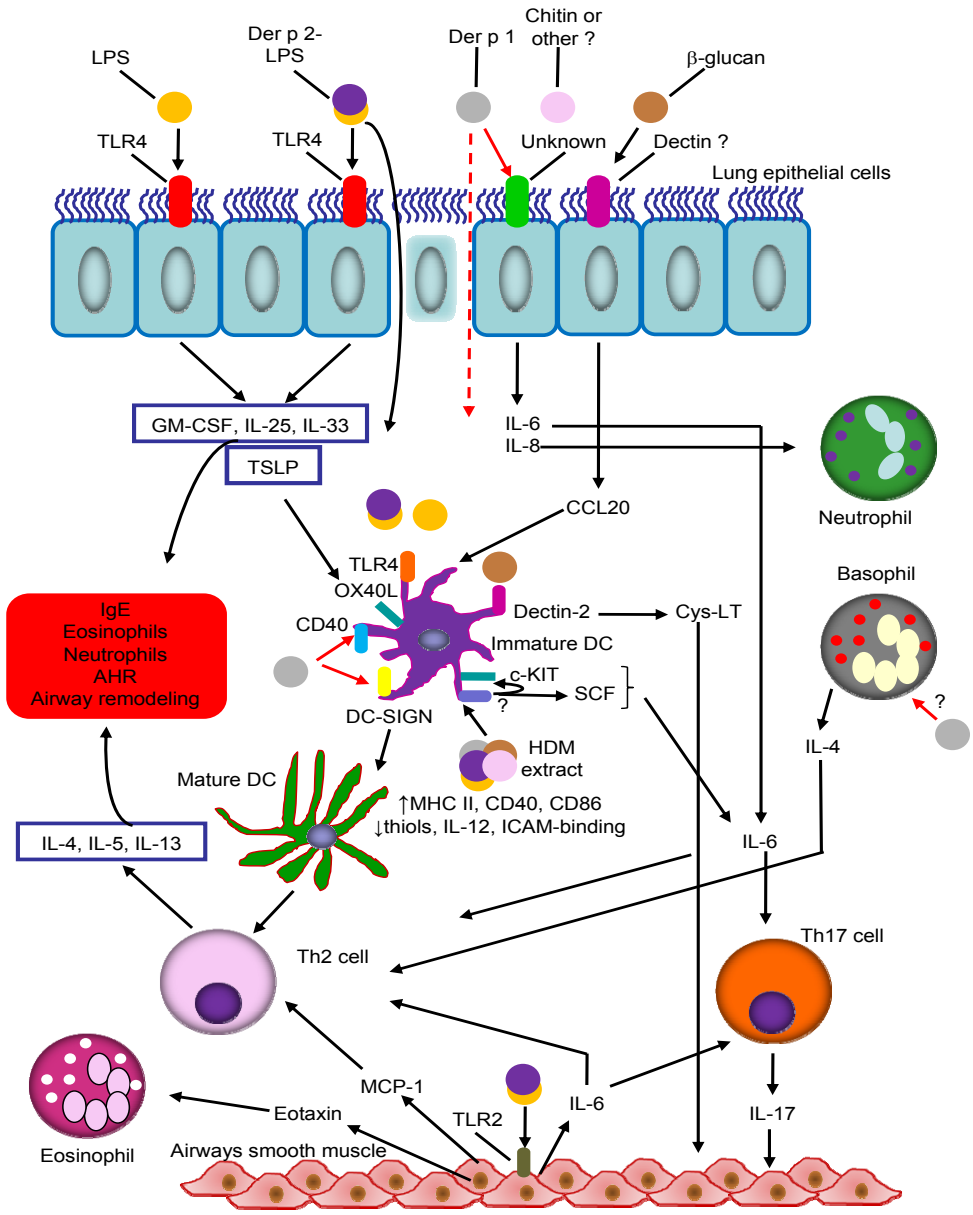

Fig. (1). Proposed model for the innate initiation of the allergic response induced by HDM.
Der p 1 and the Der p 2-LPS complex, together with adjuvant-like contaminating molecules (including LPS, β-glucan or chitin), can activate unknown protease-sensitive receptors as well as PRR expressed by epithelial cells. Altogether, these stimulations lead to the production of chemokines and cytokines that not only attract and activate DCs but promote also an influx of inflammatory leukocytes to trigger eosinophilia, neutrophilia, airway remodeling and AHR. Thanks to the increasing permeability of the epithelial barrier following cleavages of tight junction proteins by Der p 1, submucosal DCs can be also directly activated by components of HDM. There is an immediate release of Cys-LT, potent mediators of bronchial smooth muscle constriction and IL-6. Activated DC will mature and migrate to mediastinal lymph nodes to present the allergen to naïve T cells. The cytokine milieu (notably TSLP, low IL-12 concentration) will drive the differentiation of naïve T cells into Th2 cells producing the cytokines IL-4, IL-5 and IL-13, the critical effectors of the salient features of asthma as the allergen-specific IgE antibody and the recruitment and activation of eosinophils. The presence of IL-6 will induce Th polarization skewing to Th17 cells which accentuate the airway pathology through notably the action of Th17 cytokines as IL-17 on the airway smooth muscles.
TLR, Toll-like receptor; CCL, CC-chemokine ligand; GM-CSF, granulocyte/macrophage colony stimulating factor; TSLP, thymic stromal lymphopoietin; Cys-LT, cysteinyl leukotriene; SCF, stem cell factor; DC-SIGN, dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin; MCP-1, monocyte chemotactic protein-1.

search should try, using purified recombinant mite allergens, to elucidate whether exposure to these individual mite allergens are able to stimulate the production of the pro-Th2 cytokines such as TSLP, IL-25, IL-33 at the level of the airway structural as well as innate immune cells. Extensive studies should also focus on the putative additional factors from HDM which could influence the outcome of the responses to HDM allergens. The characterization of environmental compounds in HDM could thus open the door to new therapeutic approaches.

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Received: May 27, 2009 Revised: June 15, 2009 Accepted: June 17, 2009