

Interplay between barrier epithelial cells and dendritic cells in allergic sensitization through the lung and the skin

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Summary

The prevalence of asthma is increasing over the years and it has become obvious that a thorough understanding of mechanisms leading to Th2 sensitization and to asthma is urgently needed to provide better ways to treat the disease. This article reviews the different players involved in the initiation of allergic reactions in the lung and in the skin, and highlights the importance of a crosstalk between antigen-presenting dendritic cells and structural cell-derived signals in this process. Our increasing understanding of these mechanisms indicates that structural cells, such as airway epithelial cells and skin keratinocytes, need to be considered as more than a simple physical barrier since they are very upstream of the entire Th2 cascade and therefore might represent crucial targets for new therapies.

KEYWORDS

asthma, dendritic cells, inflammation, lung, skin

1 | INTRODUCTION

Asthma is a chronic inflammatory airway disease that affects 300 million people worldwide. Asthma is characterized by wheezing, coughing, and chest tightness, which is caused by a combination of events in the airways such as bronchial hyperreactivity (BHR), airway wall remodeling, and airway narrowing. These symptoms can be triggered by non-specific stimuli like cold air and exercise, or, in the case of allergic asthma, by airborne allergens such as animal dander, pollen, house dust mite (HDM), or fungal spores. Allergic asthma especially starts during the early life while non-allergic (a.k.a intrinsic) asthma is more often diagnosed in adults.¹ In children who are genetically predisposed to atopy, the allergic sensitization occurs in the first years of life and is often accompanied by eczema. Interestingly, a variety of cross-sectional and longitudinal studies revealed that about 50% of children with atopic dermatitis (AD) developed allergic asthma later in life.^{2,3} This phenomenon, where clinical manifestations of

allergies occur in a typical sequence of events, ultimately leading to the development of asthma, is called the 'atopic march'. This concept is very interesting because it raises several important questions: In which tissue does Th2 sensitization to environmental allergens really occur? And what are the mechanisms underlying the process of sensitization?

Immunologically, the clinical symptoms of allergic asthma are a consequence of a dysregulated type 2 inflammation in the lung, orchestrated by cytokines such as IL-4, IL-5, IL-9, and IL-13, known to be produced by Th2 cells and group 2 innate lymphoid cells (ILC2). IL-4 drives isotype class switching in B cells, resulting in the production of allergen-specific IgE that will trigger mast cells and basophils that express the high-affinity IgE receptor FcεRI. IL-5 is involved in the development and activation of eosinophils in the bone marrow and tissues. IL-9 causes mast cell and ILC2 activation, whereas IL-13 (and to a lesser extent IL-4) induces airway hyperreactivity, goblet cell metaplasia, mucus hypersecretion, and the differentiation of alternatively activated macrophages.⁴ Although a lot is known about the role of Th2 cells and their cytokines in allergic diseases, less is known about the mechanisms of induction of Th2 sensitization. From

several experimental mouse models for allergic asthma, it has become obvious that Th2 sensitization in the lung is driven by dendritic cells (DCs) that migrate to the draining mediastinal lymph nodes (MLNs) to activate an allergen-specific CD4⁺ T-cell response.^{5,6} In the skin, there is also evidence to indicate that Th2 responses responsible for the development of AD are also driven by DCs.⁷ Over the last years, it has become increasingly clear that barrier epithelial cells (ECs) and tissue-derived factors in general have an important role in shaping the immune responses that are generated against harmless allergens.⁴ In reality, although DCs are crucial in the establishment of Th2 immunity to allergens, they cannot do this with an intimate crosstalk with tissue structural cells. In this review, we will discuss how different populations of DCs in the lung and in the skin contribute to Th2 sensitization to environmental allergens. We will also focus on the pathways used by airway ECs and skin keratinocytes to recruit and activate various players of the type-2-cell-mediated immune responses. We will discuss the importance of barrier integrity in murine models and in patients with Th2 cell-associated disorders.

2 | DCS ARE CRUCIAL TO BRIDGE INNATE AND ADAPTIVE IMMUNITY TO AEROALLERGENS

It has long been established that antigen presentation by DCs is essential for the development of immune responses to environmental triggers. Over the years, our lab has tried to elucidate the exact role of DCs in the development of allergic airway inflammation. The first reports that appointed DCs as central players in the development of allergic airway inflammation appeared almost 20 years ago. In these studies, the administration of in vitro-generated DCs pulsed with ovalbumin (OVA) in the airways of animals lead to all features of allergic asthma.^{5,8} The development of genetic tools such as CD11c-DTR, which allowed the selective depletion of DCs at different time points of the asthma protocols, confirmed that lung DCs were crucial not only for the initiation of Th2 immunity to allergens⁶ but also in the effector phase of the response,^{9,10} likely due to their capacity to attract and reactivate primed Th2 cells in the lung.^{9,11} How DCs could promote Th2 immunity is still very poorly understood. The DC-derived signals that promote Th1 and Th17 cell differentiation have been well defined. However, the pro-Th2 signals derived from DCs are totally unknown. IL-4 is the prototypical Th2-driving cytokine, but this cytokine is not produced by DCs. However, it was suggested, by our group and others, that IL-4 might be provided to DCs by accessory innate cells such as basophils or eosinophils.⁶ In 2009, this concept was challenged by a series of papers describing that FcεRI⁺ basophils were bona fide antigen-presenting cells promoting Th2 immunity by producing IL-4 upon encounter with naive T cells.^{12,13} However, we found that, in these studies, the methods used to target basophils (eg FcεRI⁺ cell depletion) also targeted a subset of lung inflammatory DCs that expressed the same marker as basophils.⁶ In our hands, and in response to HDM allergen, inflammatory DCs and not basophils were necessary and sufficient for Th2 responses to HDM.⁶

In the recent years, more emphasis has been put on trying to elucidate the specific role that different DC subsets exert in different tissues, including the lung and the skin. The use of different markers used in different tissues and different disease settings to define DCs has prompted scientists to develop a more unified nomenclature which classifies DCs mainly based on their ontogeny.¹⁴ In all tissues, DCs can be subdivided into three main subsets that depend on specific transcription factors (TFs) for their differentiation and function. Typically, plasmacytoid DCs (pDCs) are controlled by E2-2 and Id2, type 1 conventional DCs (cDC1) by BATF3, IRF8, and Id2 and type 2 cDCs (cDC2) by KLF4 and IRF4. TFs do not only determine the development but also regulate the function and the survival of DCs. As an example, IRF8 can act as a terminal selector for cDC1, meaning that IRF8 is needed to maintain their identity and survival.^{15,16} Interestingly, the lack of IRF8 in CD11c⁺ cells resulted in an altered pDC phenotype and gene expression profile, that renders them more potent in stimulating T cells, but decreased their type 1 interferon (IFN) production.¹⁶ How the lack of IRF8 specifically in DCs would affect responses to viral infections, where pDCs are known to be very important, remains to be elucidated. Another IRF family member, IRF4, has no role in early commitment to cDC2s but appears to be involved in terminal differentiation of cDC2s, as well as their survival and capacity to migrate to the lymph nodes.^{15,17–19} Over the last years, our group has tried to unravel the role of IRF4- and IRF8-dependent DC subsets in Th2 sensitization to HDM via the lung and via the skin.

2.1 | Role of lung DC subsets in Th2 sensitization to HDM

Murine lungs contain three major subsets of DCs with specific phenotype and function; pDCs, IRF8-dependent CD103⁺ cDC1, and IRF4-dependent CD11b⁺ cDC2. In addition, inflamed lungs also contain monocyte-derived DCs (moDCs), which arise from monocytes that upregulate the expression of CD11c and MHC-II.^{11,20} Although CD11b⁺ cDC2s and moDCs have different origin, these subsets share the expression of many surface proteins, which made it difficult, for a long time, to distinguish them by regular flow cytometry. Using competitive bone marrow chimeras with CCR2-deficient mice, in which monocytes fail to egress from the bone marrow, we identified that moDCs, but not CD11b⁺ cDC2s, could be characterized by the expression of two Fc receptors, namely CD64 and FcεRI.¹¹ The ability to unequivocally identify each subset of lung DC so precisely prompted us to address their function in Th2 immunity to HDM allergen. We found that mainly CD11b⁺ cDC2s were responsible for the transport of HDM to the lung draining lymph nodes, where the antigen was subsequently presented to naive CD4⁺ T cells¹¹ (Figure 1). This CD11b⁺ cDC2 subset was indispensable for the development of eosinophilic airway inflammation to HDM. The function of CD11b⁺ cDC2s in Th2 responses to HDM was further supported by another group that made use of IRF4^{fl/fl} CD11c-Cre mice, and showed that the defect in Th2 responses to the allergen was due to a migration defect in CD11b⁺ cDC2s to the draining MLNs.¹⁷ Although cDC2 were found to be the main drivers of Th2 immunity to HDM, we

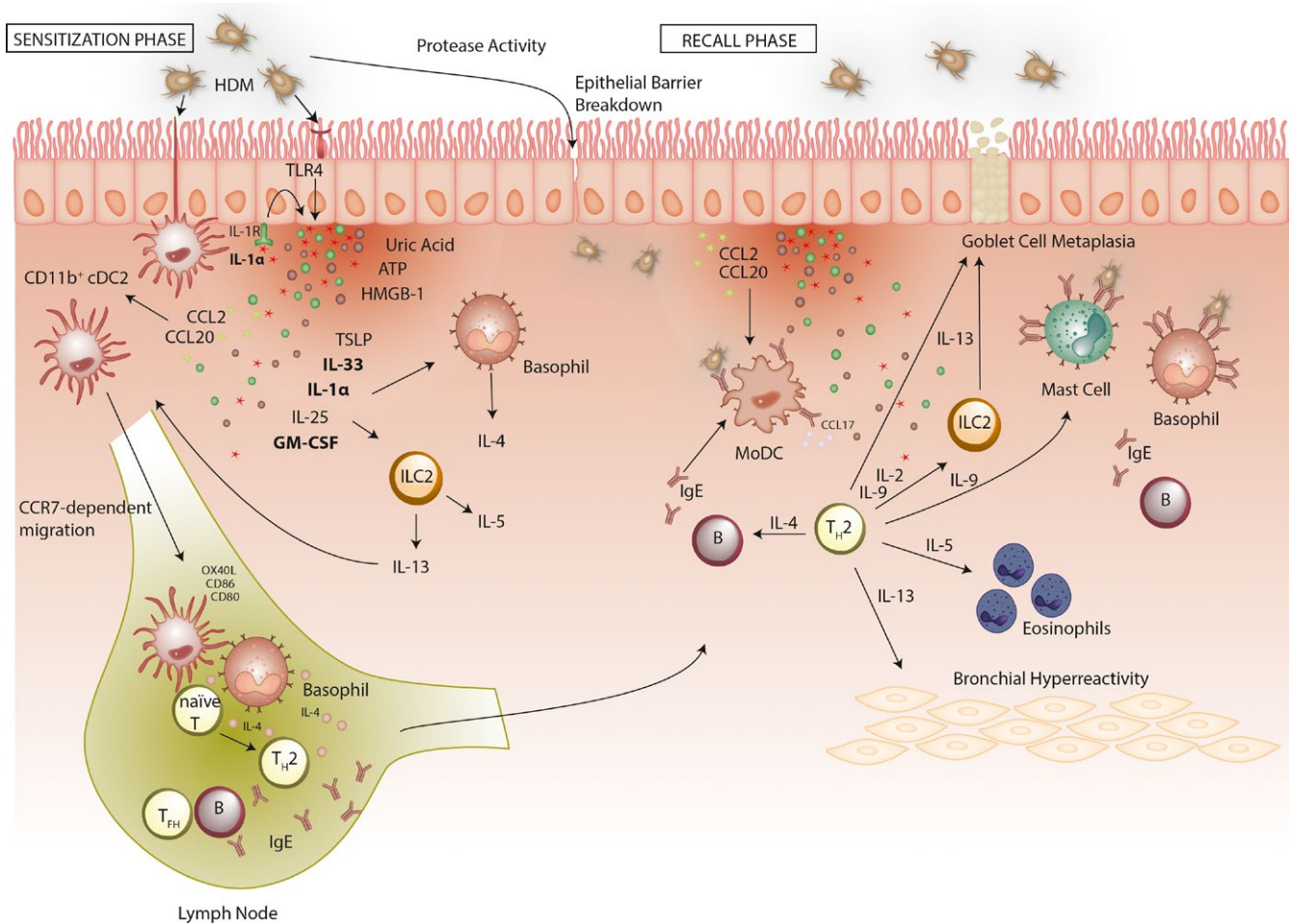


FIGURE 1 Interplay between lung epithelial cells (ECs) and dendritic cells (DCs) during the sensitization and recall phase of experimental asthma. Lung DCs scan the airway lumen by extending their dendrites through the epithelial barrier. They can be either activated directly by house dust mite (HDM) allergens or activated indirectly by HDM-induced EC-derived molecules, which are produced in a TLR4-dependent manner. In response to HDM, ECs indeed produce danger-associated molecular patterns (DAMPs; ATP, uric acid, HMGB1), chemokines (CCL2 and CCL20), and cytokines (IL-33, IL-1 α , TSLP, GM-CSF, IL-25) that recruit, activate, and skew DCs toward a pro-Th2 phenotype. This inflammatory milieu also encourages type 2 innate cells [innate lymphoid cells 2 (ILC2) and basophils] to produce cytokines such as IL-13 which stimulates the migration of lung DCs to the draining mediastinal lymph node (MLN), and IL-4 which skews differentiation of naive T cells to Th2 cells. Activated CD11b⁺ cDC2s migrate to the MLN, where they induce Th2 responses. During the effector phase (re-exposure to HDM), moDCs in the lung produce inflammatory chemokines involved in the recruitment of effector cells. In this way, mo-DCs can restimulated effector Th2 cells locally and orchestrate the pulmonary inflammatory reaction

observed that, when a very high dose of allergen was used to induce sensitization via the airways, moDCs became able to migrate to the draining lymph node and induce Th2-mediated immune response.¹¹ However, the major difference between cDC2 and moDCs was that only the latter could produce chemokines involved in the recruitment of inflammatory cells, like Th2 effector cells and eosinophils. We believe that, by showing limiting capacities to migrate to the MLN in response to HDM inhalation, moDCs might contribute to orchestrate the pulmonary inflammation developed upon re-exposure to HDM, most likely by recruiting and locally reactivating effector Th2 cells in the lungs¹¹ (Figure 1). Along this line, a report showed that the depletion of CD11b⁺ cells during the antigen recall phase hampered the chemokine-induced recruitment of Th2 cells into the airways.²¹ This CD11b-mediated effect was attributed chemokine-producing CD11b⁺ DC, but in view of our recent findings,¹¹ it is very possible

that the cell type responsible is moDCs, although this hypothesis still needs to be confirmed.

Although the role of cDC2 in promoting Th2 immunity is undisputable, the role of IRF8-dependent CD103⁺ cDC1s is less clear. These cells can efficiently transport inhaled allergen from the lung to the draining nodes, but they were unable to activate naive CD4⁺ T cells ex vivo, and they failed to induce allergic airway inflammation when adoptively transferred into naive lungs.¹¹ CD103⁺ cDC1 are mostly characterized by their capacity to produce IL-12, and it was shown recently that the constitutive expression of IL-12 by CD103⁺ cDC1 is able to suppress type 2 immunity to helminth infection. Whether this is the reason why they would not be able to induce sensitization to HDM remains to be proven. Moreover, this specific subset of DCs has also been attributed tolerogenic functions as they can induce Foxp3 regulatory T cells via the upregulation of retinaldehyde dehydrogenase

2 (*Aldh1a2*), a key molecule for the production of retinoic acid.²² This retinoic acid functions as a cofactor for the TGF-mediated upregulation of Foxp3 in naive T cells.²² Interestingly, mice lacking CD103⁺ cDC1 showed an exacerbated Th2 response to parasitic infection and a break of inhalation tolerance to inhaled OVA.^{22,23} These data clearly indicate that cDC1 have anti-inflammatory properties and suppress Th2 responses. In our hands, the depletion of this subset at the time of HDM sensitization failed to exacerbate asthma features.¹¹

pDCs have a well-known role in antiviral immunity in many organs, but their role in allergic diseases is somewhat less clear. pDCs are equipped with specific PRRs that recognize viral antigens and react immediately by producing type 1 and type 3 IFNs.²⁴ A decade ago, our group and others have shown that pDCs in the lung were essential to induce inhalation tolerance to harmless antigens like OVA,^{25,26} showing their anti-inflammatory potential under steady-state conditions. Intriguingly, this suppressive function of pDCs was also observed during the effector phase of the asthmatic response. Indeed, the selective depletion of pDCs during the challenge phase in already sensitized animals enhanced airway inflammation.²⁷ Whether these anti-inflammatory properties of pDCs are inherent in mouse models or whether they are present in humans needs to be carefully addressed. Clinical studies in asthmatic patients reported aberrant numbers of peripheral pDCs and this was strikingly related to age. In children at risk of developing asthma later in life, the number of pDCs was reduced compared to “healthy” children, suggesting that higher pDC numbers in infancy appear to be protective against asthma inception.²⁸ However, in asthmatic adults, the correlation is inversed, and their number of pDCs was higher than in healthy controls. One common feature shared by pDCs from asthmatic children and adults is certainly their impaired capacity to produce type I IFNs in response to viruses.^{29,30} This aberrant IFN-I response by pDCs might explain why respiratory viral infections induce exacerbations in asthmatic adults and children.

2.2 | Role of skin DC subsets in Th2 sensitization to HDM

Like all other barrier tissues, the skin harbors DCs that act as sentinels and constantly scan their surroundings for the presence of invading pathogens. The murine skin contains at least four major subsets of DCs. Langerhans cells (LCs) are the only DCs that reside in the epidermis but they are also found in the dermis when they transit to the draining LN. The dermis contains cDC1s and cDC2s that are, just like in the lungs, specialized in presentation to CD8⁺ and CD4⁺ T cells, respectively. Additionally, moDCs are abundantly present in steady-state dermal tissue. There are no or few pDCs in the skin during steady state, but they can be recruited upon cutaneous viral infections or injury that requires wound healing processes.³¹

In the lung, IRF4-dependent cDC2s have been shown to drive both Th2 and Th17 immunity.^{17,19,32,33} In the skin, the IRF4-dependent cDC2 subset is a heterogeneous population which is composed of two populations: CD11b⁺ cDC2, that also express the C-type lectin Mgl2 (CD301b) and programmed death ligand-2 (PD-L2),^{32,34} and a

population of so-called “double negative” (DN) DCs that lack high expression of CD11b and CD103.^{35,36} Many studies have addressed the role of skin cDC2 and have found that this subset could induce Th2 immunity.^{17,32,34,37,38} One of the possible explanation for this is that CD11b⁺ cDC2s are highly responsive to the keratinocyte-derived cytokine TSLP, which can endow them with pro-Th2 capacities.^{37,39} One caveat with all these studies is that none of them had been able to study the specific role of each subpopulation contained in the skin cDC2 subset (namely CD11b⁺ and DN cDC2). Recently, Tussiwand et al.⁴⁰ discovered that cutaneous IRF4-dependent cDC2s could be unambiguously subdivided based on the expression of another transcription factor, Kruppel-like factor 4 (KLF4), which expression, in the skin, is restricted to DN cDC2s. These authors describe a very clear division of labor between KLF4-dependent DN cDC2s, which would be responsible for mounting Th2 immunity, and KLF4-independent CD11b⁺ cDC2s which would be responsible for mounting Th17 immunity. We recently found that such specialized functions of DCs really depended on the type of antigen used. When we applied HDM on the skin of mice to induce Th2 sensitization epicutaneously, we found that IRF4-dependent cDC2s were indeed required for Th2 response to HDM.³⁸ However, in response to HDM allergen, both KLF4-dependent DN and KLF4-independent CD11b⁺ cDC2s induced a strong Th2 response when transferred intradermally into naive mice (Figure 2). We did not observe any increase in Th17 responses in mice that received HDM-loaded CD11b⁺ cDC2s compared to the mice that received HDM-loaded DN cDC2s. Our data show that the division of labor described by Tussiwand et al. is not solely restricted to KLF4 expression by cDC2, but it might also involve signals, derived from the microenvironment and induced by different types of antigens⁴¹ that might steer DCs toward a specific type of immune response.

Dermal cDC1s are characterized phenotypically by the surface marker XCR1, which is uniformly expressed on cDC1s in all lymphoid and non-lymphoid tissues.⁴² Langerin (CD207) expression is, however, restricted to a subset of cDC1s from the lungs and skin.^{16,38,43} cDC1s in the skin are either CD103⁺ or CD103[−] and like in other tissues, the CD103⁺ cDC1s have been implicated in cross-presentation of keratinocyte-derived self-antigen.⁴⁴ Additionally, dermal cDC1s have been associated with priming of cytotoxic T-lymphocytes and Th1 responses against cutaneous *C. albicans* infection.⁴⁵ In a Th2 model for allergic airway inflammation, we found that Langerin⁺ cDC1s are dispensable for epicutaneous sensitization to HDM.³⁸ HDM-loaded cDC1s were not able to induce Th2 responses when transferred intradermally into naive mice. The future generation of genetically manipulated mouse strains should help unravel the importance of different cDC1 subsets in type 2 immunity, induced via the skin or other organs. As mentioned above, IRF8 plays a crucial role at different stages of DC development, and we recently showed that it functions as a ‘terminal selector’ of cDC1s, regulating its cellular functions and maintains its cDC1 identity.¹⁶ Therefore, this mouse line might prove very useful to address the exact role of cDC1 in allergic diseases.

In addition to conventional DCs, moDCs are found in the dermis during steady-state conditions.⁴⁶ They arise from blood-born Ly6C^{hi} monocytes and are recruited to the dermis in a CCR2-dependent

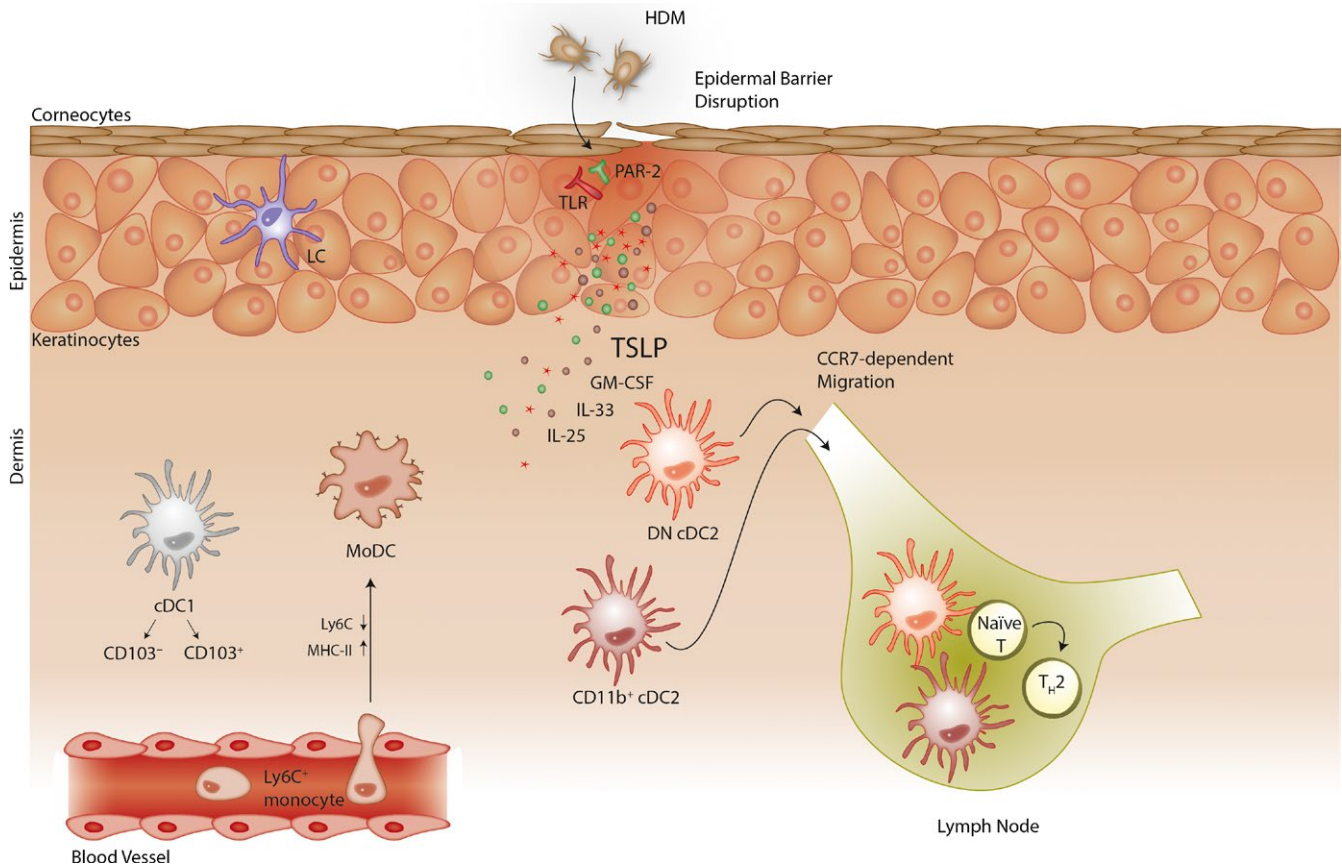


FIGURE 2 Epicutaneous sensitization to house dust mite (HDM). Langerhans cells (LCs) are the only dendritic cells that reside in the epidermis, whereas the dermis contains cDC1s (either CD103⁻ or CD103⁺), DN cDC2s, CD11b⁺ cDC2s, and monocyte-derived DCs (moDCs). HDM can be recognized by pattern recognition receptors (TLR, PAR-2) on keratinocytes and induce the production of typical type 2 immune cytokines (TSLP, GM-CSF, IL-33, IL-25). This inflammatory environment results in the activation and CCR7-dependent migration of both cDC2 subsets (DN and CD11b⁺) to the skin-draining lymph node where they mount a Th2 response. Epidermal barrier dysfunction enhances Th2 responses but are not absolutely required for the induction of Th2 immunity to allergens via the skin

manner, where they gradually upregulate MHC-II and downregulate Ly6C.⁴⁶ We have recently shown that they were capable of taking up epicutaneously applied HDM³⁸ but as described for other organs,^{11,45,46} moDCs in the skin have a poor migratory capacity.³⁸ However, just like in the lung, their migration can be induced by the application of high doses of HDM on the skin [our unpublished findings and Ref. (11)]. Because of their poor migratory properties, and based on our previous findings in the lung, it is tempting to speculate that moDCs might be dispensable during epicutaneous sensitization to HDM and that they would be more important in driving response during the effector phase of allergic reactions. The lack of tools to specifically deplete moDCs has impeded researchers to confirm or disprove this hypothesis, but data obtained in humans have evidenced a role for these cells during allergic diseases. Indeed, ex vivo-cultured moDCs from AD patients express measurable amounts of the C-type lectin receptor DC-SIGN that recognizes and promotes uptake of allergens such as HDM.⁴⁷ Der p 2, one of the major allergen of HDM, stimulated these cells via DC-SIGN to produce pro-inflammatory cytokines that skewed naive T cells toward a Th2 or Th22 phenotype. Furthermore, DCs in skin biopsies from patients with AD expressed much higher levels of DC-SIGN compared to healthy controls,⁴⁷ although in this study, it was not clear

whether DC-SIGN was restricted only to moDCs or was expressed by some cDCs. It might therefore be interesting to study more precisely how DC-SIGN and moDCs contribute to allergic skin inflammation.

The skin is the only organ that contains LCs, additional antigen-presenting cells with particular properties. LCs reside in the epidermis and origin from yolk sac-derived myeloid precursors.⁴⁸ Given their anatomical localization, they are the first line of defense against antigens or pathogens that come in contact with the skin surface. Their dendrites reach the extracellular space and are closely attached to neighboring keratinocytes by tight junctions (TJ) to guarantee preservation of the epidermal barrier. LCs are constantly traveling to the skin-draining LNs, a process which is called homeostatic migration and involves phenotypic maturation such as upregulation of CCR7 and downregulation of E-cadherin in order to detach from keratinocytes. The disruption of keratinocyte TJ is believed to be involved in the potential tolerogenic phenotype of LCs via the release of β -catenin.⁴⁹ LCs are long-lived cells that are radioresistant and self-renewing. In steady state, they undergo a low-rate proliferation in order to repopulate the epidermal LCs pool. During severe skin injury, however, an inflammatory environment with concomitant release of chemokines results in replenishment of the empty epidermal niche by bone

marrow precursors in a CCR2- and CCR6-dependent way.⁴⁸ The classification of LCs as DCs or macrophages is a matter of debate. Contrary to DCs, they are long-lived cells from embryonic origin and express the macrophage marker F4/80, but unlike tissue macrophages, they are constantly migrating to draining LNs where they can present antigen via MHC-II molecules.^{48,50} In fact, a recent paper revealed a double identity for LCs by means of a very elegant lineage tracing method.⁵¹ LCs were found to be a unique subset that was lineage traced for *Mafb*, indicating macrophage progeny, but they simultaneously expressed *Zbtb46*, which is shared by all classical DCs.⁵¹ Until now, the precise role of LCs during sensitization to allergens is still under debate. Although LCs have been shown to play a role in the epicutaneous sensitization to protein antigen OVA,⁵² we found that LCs were dispensable for sensitization to HDM via the skin.³⁸ Furthermore, we did not observe any Th2 response when we transferred HDM-loaded LCs into the skin of naive mice.³⁸ The discrepancy between these two findings might be due to the different methods used to induce epicutaneous sensitization. The first model employed the inert protein OVA applied on a shaved skin subjected to different rounds of tape stripping.⁵² In our experiments, HDM allergen was applied on the skin in the absence of any shaving and any tape stripping. As a matter of fact, we have found that the shaving of the skin enhanced Th2 immunity to HDM.³⁸ This action combined to tape stripping might disturb the skin barrier integrity, as evidenced by an increase in transepidermal water loss.³⁸ This increased skin permeability along with the release of pro-inflammatory factors induced by the procedure might be at the heart of the difference observed in response to OVA and HDM, likely allowing LCs to induce Th2 immunity to allergens when applied on a "damaged" skin. Although, in our experiments, LC depletion did not affect Th2 immunity to HDM applied epicutaneously, we observed that the absence of LCs during epicutaneous sensitization to HDM resulted in an increased Th2 cytokine production by the MLN cells during the HDM allergen recall phase in the lungs.³⁸ These findings are in line with results from other groups showing that the constitutive lack of LCs was found to enhance cutaneous sensitization to haptens.^{53,54} These data might be explained by the emerging consideration that LCs might have an important function in peripheral tolerance.⁵⁵ Indeed, LCs have been reported to induce the expansion of FOXP3⁺ regulatory T cells and the deletion of allergen-specific CD8⁺ T cells.^{56,57} The role of LCs in skin allergic diseases still remains, very unclear. This should be investigated more thoroughly in mouse models and, certainly, the translation of these findings to human patients remains to be addressed.

3 | BARRIER EPITHELIAL CELLS IN THE LUNG PLAY AN IMPORTANT ROLE IN SHAPING THE IMMUNE RESPONSE TO ALLERGENS

Because of their anatomical site at the border between the external environment and the internal organism, ECs in the lung and in the skin operate as a first line of defense against invasion of pathogens or

environmental insults. Besides their important function of physical barrier, it is only 15 years ago that Holgate et al.⁵⁸ have proposed a critical role for lung ECs in the immunology of asthma. Since these initial studies, many research efforts have confirmed that airway ECs actively contribute to the immune responses to allergens or microbes, mostly by interacting with different neighboring immune cells. In the lung, ECs express a wide variety of pathogen recognition receptors (PRRs) that can be activated by inhaled antigens, and the triggering of PRRs on ECs stimulates ECs to secrete danger-associated molecular patterns (DAMPs), alarmins, chemokines, cytokines, and other immune mediators. Another way by which ECs in the body can be activated is through the disruption of the barrier function. As such, some allergens contained in HDM or fungi are proteases, and have been shown to cleave TJs between ECs, thus favoring the access of antigens to immune cells located in the vicinity of ECs.^{59,60} However, whether this disruption of TJ is a general mechanism required for Th2 sensitization remains unclear.

3.1 | Triggering of pattern recognition receptors on lung epithelial cells is required for Th2 immunity to inhaled allergens

ECs in the lungs are constantly exposed to harmful insults. Therefore, it is not surprising that these cells express many PRRs leading to intracellular signaling pathways designed to help deal with the insult. Allergens, considered as harmless for most of humans, can induce an exaggerated Th2 response that will be detrimental in the case of allergic patients. For many years, we have tried to understand how Th2 sensitization to inhaled allergens was initiated in the airways, and whether some PRRs would be critical in this process. Using a mouse model of allergic asthma driven by HDM, we found that Th2 sensitization to HDM depended on Toll-like receptor 4 (TLR4) signaling in lung structural cells⁶¹ (Figure 1). Radiation-induced bone marrow chimeras, lacking the expression of TLR4 on structural cells but not on hematopoietic cells, failed to mount proper Th2 immunity to the allergen. Mechanistically, we found that, in the lungs of these chimeric mice, there was very little production of innate pro-allergic cytokines (TSLP, IL-33, IL-25, IL-1 α , and GM-CSF) in response to HDM, compared to mice that expressed TLR4 on structural cells. As a consequence, DC maturation was impaired and the typical Th2-mediated airway inflammation, which occurred upon airway HDM challenges, was completely prevented.⁶¹ The central role of TLR4 in the initiation of Th2 immunity to environmental allergens has, since, been shown by several groups,^{62,63} and the specific requirement of TLR4 on lung ECs in Th2 responses has recently been confirmed in a study using transgenic mice that conditionally lacked TLR4 in ECs.⁶⁴ How exactly allergens can trigger TLR4 signaling is currently unknown. It has recently been suggested that HDM could induce the upregulation of TLR4 expression in ECs, leading to a break in inhalational tolerance to aeroallergens, and thus favoring sensitization.⁶⁵ Also, Trompette et al.⁶⁶ showed some years ago that TLR4 signaling could be initiated by the major HDM allergen Der p 2, which mimics the lipopolysaccharide (LPS)-binding subunit MD-2, leading to the facilitation of TLR4 signaling. Similarly, HDM allergen Der f 2 can bind directly to TLR4 and elicit pro-inflammatory cytokine production in human bronchial ECs.⁶⁷

Since a substantial proportion of HDM allergens are proteases, attention has also been given to the involvement of protease-activated receptors (PARs) on ECs. Among the four PAR family members, only PAR-2 can be activated by serine proteases that are present in HDM. Although the presence of PAR-2 on bronchial ECs was reported to be crucial for the response to some allergens like mold, cockroach or fungi,^{68–70} PAR-2 function in Th2 immunity to HDM via the lungs is more debatable.^{71,72} Recently, new light was shed on the role of PARs in asthma. Indeed, HDM proteolytic allergens like Der p 1 do not activate PAR-2, but they were shown to cleave prothrombin to generate the clotting factor thrombin, which can in turn activate PAR-1 and PAR-4 on ECs. Subsequently, ECs produce intracellular reactive oxygen species (ROS) via extracellular release of ATP.⁷³ Extracellular ATP is considered as an endogenous danger signal which levels are elevated in asthmatic patients, and ATP has been shown to trigger Th2-associated allergic airway inflammation by activating lung DCs.⁷⁴

3.2 | Endogenous danger signals released by lung epithelial cells function as inducers of Th2 immunity to inhaled allergens

We have previously reported that DAMPs like ATP and uric acid were induced in lung ECs upon a single exposure to HDM,^{74,75} and by a wide range of allergens.^{76,77} These DAMPS were able to activate DCs to mount Th2 responses, and uric acid stimulated the production of typical Th2 skewing cytokines by ECs in a TLR4-dependent manner.^{75,76} A recent paper demonstrated that particulate matter, known to facilitate allergic sensitization, could also induce uric acid production by airway ECs, but in a TLR4-independent manner.⁷⁸ Although particulate matter was able to activate an innate response in ECs via NLRP3/IL-1 β inflammasome pathway, this pathway was completely dispensable for the facilitation of allergic sensitization.⁷⁹ In agreement with these findings, we found that the type 2 immunity-promoting effect of uric acid was not dependent on NLRP3/IL-1 β signaling, nor was it affected by the lack of ASC or caspase-1.⁷⁵ Instead, we reported that HDM stimulated a very rapid EC-derived IL-1 α release, which then triggered IL-1R on radioresistant cells (mostly ECs), leading to the induction of Th2 immunity to HDM.⁸⁰ This autocrine action of IL-1 α stimulated the production of innate pro-Th2 cytokines by ECs, like GM-CSF and IL-33.⁸⁰ Also, downstream of IL-1 α , HDM can induce the secretion of high mobility group box 1, another alarmin associated the induction of allergic disorders through its receptor, receptor for advanced glycation end-products (RAGE).^{81–83} More specifically, the absence of RAGE expression resulted in a deficit of ECs to produce innate pro-Th2 cytokines.^{81,84}

3.3 | Innate pro-Th2 cytokine production by lung epithelial cells drive Th2 immunity to inhaled allergens

Aeroallergens, like HDM, trigger airway ECs to produce prototypical type 2-promoting cytokines such as IL-33, thymic stromal lymphopoietin (TSLP), and IL-25 (Figure 1). These cytokines play an instructive role for DCs to provoke adaptive Th2 responses and license activation

of innate type 2-associated cells like ILC2s, basophils, mast cells, and eosinophils.⁸⁵ We also identified GM-CSF as one of these HDM-induced EC-derived cytokine that was crucial for the initiation of allergic airway inflammation. In response to HDM exposure, GM-CSF was responsible for the activation of lung DCs, and contributed to endow them with Th2-inducing properties.^{80,86,87}

TSLP activates STAT5 in DCs, which induces signaling pathways leading to the upregulation of CD80, CD86, and OX40L costimulatory molecules and CCL17 chemokine production.⁸⁸ Although TSLP has been reported to promote Th2 responses by DCs ex vivo,⁸⁹ we and others found that experimental allergic airway inflammation induced by a low-dose HDM administration in the lungs did not depend on TSLP.^{80,90} In our hands, we only found TSLP dependency in vivo when we sensitized wildtype and TSLPR^{-/-} mice with an extremely high dose of HDM.⁸⁰ In line with this, in a model of chronic exposure to high doses of HDM, TSLPR^{-/-} mice failed to develop allergic airway inflammation,⁹¹ clearly showing that TSLP became important in response to high doses of inhaled allergens. However, these data obtained using TSLP-deficient mice have to be considered with caution. Indeed, in another chronic model of high-dose HDM exposure, when TSLP-blocking antibodies were injected 4 weeks after sensitization, there was no effect of any of the allergic asthma features.⁹² All these data indicate that TSLP might be important only during the initiation of Th2 immunity to HDM and only upon exposure to high doses of allergen. In patients, the link between augmented levels of TSLP and allergic diseases, like AD, allergic rhinitis, and asthma, has been well established.^{89,93,94} However, the findings obtained in patients and in animal models are conflicting. A recent clinical trial using a TSLP-blocking antibody showed a reduction in allergen-induced bronchoconstriction and in clinical features of asthma.⁹⁵ It therefore becomes of importance to further investigate where and when exactly TSLP acts in allergic diseases, and in which type of allergic patients TSLP is relevant in order to aid clinical trials and provide better-targeted therapies.

IL-33 is another EC-derived cytokine that is linked to asthma in patients. Serum IL-33 levels are increased in asthmatic patients compared to healthy controls, and genetic variations in the *IL1RL1* gene, which encodes the IL-33 receptor (ST2) as well as the decoy receptor (soluble ST2), is strongly associated with allergic diseases.^{96–99} Many research papers have designated this cytokine as a central player in the response to HDM allergen through its capacity to activate group 2 ILC2s.^{65,96–100} ILC2 exposed to IL-33, in turn, produce IL-13, which is required to help lung DC migration to the draining lymph nodes.¹⁰¹ Recently, we also highlighted the importance of the IL-33 axis in the lungs of neonatal mice.¹⁰² More specifically, we showed that the IL-33 axis is spontaneously activated in lungs of neonatal mice during the alveolarization phase, which occurs in humans until the age of 3 years. Increased levels of IL-33 produced by lung ECs, during this phase, contribute to the accumulation of Th2-associated innate immune cells such as ILC2s, mast cells, basophils, and eosinophils. The administration of HDM during this phase of postnatal alveolarization further enhanced IL-33 production, activated OX40L expression on neonatal cDCs, which promoted Th2 cell skewing and allergic airway inflammation.¹⁰² This window of lung alveolarization might therefore constitute

a time window during which babies are more susceptible to become sensitized to environmental allergens. These findings also certainly help to explain why polymorphisms in the *IL1R1* gene are associated with childhood asthma,¹⁰³ and emphasize how important it might be to further untangle the early immune responses in the lung that render children susceptible to allergic diseases.

IL-25 is a cytokine from the IL-17 family and is known to activate innate type 2 responses in the gut and the lung.¹⁰⁴ Very recent papers using reporter mice revealed that tuft cells, which are particular mucosal ECs in the gut and in the trachea, were the only producers of IL-25. It has recently been established that IL-25 was able to stimulate ILC2s in the lamina propria to produce IL-13 which will in turn stimulate mucus production by goblet cells.¹⁰⁵ Although these pathways might also play a role in allergic airway inflammation, the specific role of IL-25 in asthma still needs to be clarified. We recently found that EC-derived IL-25 cooperates with IL-21-producing CD4⁺ T cells to induce lung eosinophilia and expression of muc5AC, which is indicative of IL-13-induced mucus production.¹⁰⁶ The finding that these synergistic effects were absent in mice lacking T cells and that the IL-17RB is expressed on Th2 cells suggests that this combination promotes cytokine production by Th2 cells in the lung.¹⁰⁶

3.4 | The threshold of lung epithelial cell activation regulates the induction of Th2 responses to inhaled allergens

The threshold of EC activation seems to be influenced by the degree of exposure to the external environment. One mechanism through which ECs can reset their activation threshold might be through the regulation of PRR expression on these ECs. For instance, it has been shown that environmental triggers like cigarette smoke, diesel exhaust particles, and infection with Respiratory Syncytial Virus (RSV), known to facilitate or exacerbate allergic inflammation,^{107–109} can upregulate TLR4 expression on airway ECs.^{110,111} Furthermore, viral infections like RSV and rhinovirus can promote the production of pro-inflammatory cytokines in the airway epithelium.^{112,113} In this way, these triggers might facilitate asthma exacerbations that depend on the activation of type 2 immunity.¹¹⁴ Conversely, exposure to other environmental triggers have been linked to a protection from asthma development. This has been the basis of the "Hygiene Hypothesis". From epidemiological studies, it appears that children exposed to high levels of endotoxin or to a farming environment are strongly protected from the development of allergy and asthma.^{110,111,115–118} The protection induced by these triggers has been shown to be partially mediated by regulatory T cells.¹¹⁹ However, it might also be that other mechanisms account for the effect of farm dust and endotoxin. We found recently that HDM-induced asthma features in mice could be prevented by the chronic administration of low doses of endotoxin or farm dust before sensitization to HDM.¹²⁰ The pretreatment with endotoxin or farm dust hampered the potency of DCs to migrate to draining LN and to induce Th2 responses to HDM. This appeared to be a consequence of a lack of production of instructive innate pro-Th2 cytokines like CCL20, IL-33, and GM-CSF, which are normally

produced by airway ECs as a reaction to HDM insult. We reasoned that what endotoxin and farm dust have in common might be their capacity to trigger PRRs in lung ECs. Most PRR pathways activate NF- κ B signaling and it has recently been shown that transgenic mice with a constitutively active NF- κ B in airway ECs showed a break in inhalation tolerance and became susceptible to allergen sensitization.¹¹⁹ NF- κ B activation induced by environmental factors therefore needs to be tightly controlled in order to avoid deleterious effects. Such controls generally involve negative regulators, such as TNFAIP3 (a.k.a. A20), the role of which is to terminate this pathway, thus avoiding hyperstimulation of cells.¹²¹ We have shown that endotoxin and farm dust exposure increased A20 expression in lung ECs, which altered the capacity of ECs to respond to HDM allergen.¹²⁰ Interestingly, mice that lacked A20 expression specifically in airway ECs and subjected to HDM-induced allergic airway inflammation failed to be protected by the pre-exposure to endotoxin or to farm dust.¹²⁰ Our work, therefore, provides the first evidence that part of the mechanisms explaining the hygiene hypothesis are very upstream of the immunological cascade, and involve the activation threshold of barrier ECs.

4 | ALLERGEN SENSITIZATION VIA THE SKIN: MORE THAN A DECREASED EPIDERMAL BARRIER FUNCTION

Although in the lung, the role of epithelial barrier dysfunction in the development of Th2 immunity is still debatable, in the skin, there is strong evidence linking both phenomena. The epidermal barrier of healthy skin consists of different layers that contribute to its barrier function and prevent water loss and invasion of allergens and bacteria.¹²² Therefore, it is not very surprising that Genome Wide Association studies revealed that epidermal barrier defects, caused by dysfunction of important TJ or scaffolding proteins, are correlated with allergen sensitization in human diseases.¹²³ In fact, a recent retrospective PubMed search study disclosed that the most significant risk factor for the development of AD is null mutations in the filaggrin (*Flg*) gene, which codes for a protein with multiple roles in the maintenance of epidermal barrier function.¹²⁴ The importance of the epidermal barrier function in sensitization to allergens was emphasized by numerous experimental models where proteins involved in barrier integrity were genetically mutated [reviewed in (123)]. *Flg* mutant mice on a proallergic Balb/C background developed spontaneous AD-like inflammation.¹²⁵ Additionally, keratinocyte-specific ablation of corneodesmosin, a structural protein of corneodesmosomes responsible for intercorneocyte adhesion, resulted in a breakdown of the epidermal stratum corneum and AD-like inflammatory skin response in mice.¹²⁶ Subsequently, systemic sensitization seemed to be facilitated when the allergen was exposed on a compromised skin barrier.^{125,127} Therefore, many experimental models of epicutaneous sensitization induce barrier disruption by tape stripping or shaving prior to exposure to protein antigen OVA or aeroallergens.^{128–130} We have recently found that epicutaneous sensitization to HDM could occur via a totally intact skin and lead to the development of allergic asthma

upon re-exposure of the mice to HDM via the lungs.³⁸ This was a very surprising, yet interesting, finding in view of the fact that filaggrin mutations have been shown in only 20% of asthmatic patients with AD.¹³¹ This suggests that, although defects in skin barrier functions might predispose some people to sensitization and asthma development, this might not be the sole explanation for it.

4.1 | Activated keratinocytes: Crucial players in skin type 2 immunity to allergens

Since epicutaneous sensitization to HDM did not require severe barrier disruption,³⁸ our findings support a theory that the keratinocytes in the epidermal barrier, just like airway ECs, might also actively contribute to the immune response by secreting immune mediators. The prototypical keratinocyte-derived pro-Th2 cytokine is TSLP (Figure 2). TSLP was shown to be induced upon barrier disruption¹³² but is also actively upregulated ex vivo in human keratinocytes when stimulated with TLR ligands.^{133,134} In our model of epicutaneous sensitization to HDM, we recently found a strongly reduced Th2-mediated eosinophilia in the lungs of TSLP receptor-deficient mice, indicating TSLP dependency without serious barrier disruption (J. Deckers, unpublished data). Moreover, a recent clinical study showed that HDM application induced TSLP expression in skin biopsies of non-lesional skin of patients with AD while this expression was not particularly upregulated in their untreated lesional skin biopsies.¹³⁵ Although TLR ligands are abundant in the HDM extracts and could therefore activate PRRs, other components might also stimulate keratinocytes to produce pro-inflammatory cytokines. In vitro studies showed that mite serine proteases induced cytokine production by human keratinocytes via activation of the protease-activated receptor-2 (PAR-2).¹³⁶ Moreover, PAR-2 activation in keratinocytes enhances the TSLP production in *Flg* mutant mice¹³⁷ and the PAR-2-mediated secretion of TSLP has been shown to trigger itch via sensory neurons.¹³⁸ Although these mechanisms might contribute to the clinical features of AD, other experimental models indicate that PAR-2 activation or protease activity is dispensable for allergic sensitization. PAR-2-deficient mice were not protected to HDM sensitization via the lungs⁷² and the model allergen papain was able to induce Th2-mediated response independent of its cysteine protease activity.¹³⁹ Accordingly, we found that the proteolytic activity in HDM extracts was not crucial for sensitization via the skin.³⁸ Since the barrier of epidermal TJs is size limiting, there is also the possibility that some HDM allergens could be small enough to penetrate and directly activate LCs in the epidermis or DCs in the dermis.¹⁴⁰ One should also keep in mind that animals have generally higher skin permeability than humans, so carefulness is required when drawing conclusions from animal models.¹⁴¹

4.2 | Severe barrier disruption induces a Th17 component in the response to allergens

The idea that the phenotype in atopic diseases relies solely on Th2 cells has long been refuted.¹⁴² Increased levels of IL-17 are described in the sputum of asthmatic patients and this cytokine has been linked

to the severity of the disease, probably by creating a favorable milieu for neutrophil accumulation.^{143,144} Wang et al.¹⁴⁵ described a subset of resident memory Th2 cells that were capable of producing IL-17 and a recent report underscored the fact that patients with AD might exhibit a Th2/Th17 phenotype.¹⁴⁶ HDM allergic patients carry Der p1- and Der p2-specific T cells that appeared to have a Th2, Th17, or Th2/Th17 phenotype.¹⁴⁶ Research from the Geha group and others reported the induction of a Th17 response in models of epicutaneous sensitization to protein antigens.^{147,148} Strikingly, these models included skin barrier disruption by tape stripping prior to epicutaneous antigen exposure. On the opposite, epicutaneous sensitization to HDM, in the absence of a major barrier disruption, was associated with a strong Th2 response without any Th17 component.³⁸ Yoon et al.¹⁴⁹ revealed that tape stripping induced IL-23 secretion by keratinocytes, which is a critical cytokine for Th17 polarization. Mechanical injury provoked the expression of S100A8 and S100A9 in keratinocytes, which are typical DAMPs that act as endogenous TLR4 ligands. This TLR4-mediated activation of keratinocytes appeared to be indispensable for IL-23 induction and subsequent activation of the Th17 axis.¹⁴⁹ These DAMPs were also increased in AD lesions from patients¹⁵⁰ and appeared to be induced when keratinocytes were treated ex vivo with HDM or IL-17A.¹⁵¹ This indicates a feed forward loop where IL-17A acts as an amplifier to aggravate AD features. The presence of a decreased epidermal barrier induced by mechanical injury, genetic mutations in epidermal barrier components or via excessive skin scratching, is certainly associated with a Th17 response, and might therefore explain why children with filaggrin mutation have more severe asthma.¹⁵²

Additionally, dysbiosis of skin microbiota has been proposed to be involved in the Th17 induction.¹⁵³ It is known that patients with AD are more susceptible to colonization with *Staphylococcus aureus* and this dysbiosis subsequently aggravates skin inflammation.¹⁵⁴ However, tape stripping under germ-free conditions elevated IL-23 production by keratinocytes to a same level as in specific pathogen-free conditions, suggesting that the presence of microbiota is not necessary for Th17 induction.¹⁴⁹ Nevertheless, experimental models were able to demonstrate a direct influence of microbiota on CD4⁺ T-cell skewing upon epicutaneous sensitization.¹⁵⁵ The IL-23/Th17 axis that was activated by severe skin barrier dysfunction could be counter-regulated when skin bacteria were killed by topical treatment with antibiotics.¹²⁶ The positive effects of probiotics such as *Lactobacillus rhamnosus* to prevent the onset of atopic disease in children at risk has been described in clinical trials and is attributed to the induction of regulatory T cells.¹⁵⁶ In an experimental model of epicutaneous sensitization, these probiotics induced Tregs that were able to counteract Th2/Th17 responses.¹⁵⁷

Although it is tempting to target mediators aggravating AD severity, caution is recommended when targeting the IL-23/Th17 axis. IL-23 that is produced by activated keratinocytes and DCs induces the production of IL-22 by innate $\gamma\delta$ T cells, which in turn stimulates the production of antimicrobial peptides by keratinocytes.¹⁵⁸ Besides, IL-22, together with IL-17A and IL-17F, are crucial for the expression of neutrophil attractants Cxcl1 and Cxcl2 in skin and the phagocytic capacity

of neutrophils and their production of ROS are essential at the site of inflammation for clearance of *S. aureus*.¹⁵⁸

5 | CONCLUSION

Although many immune cells have an important function in the effector phase of allergic airway inflammation, there is a huge body of evidence that the interaction between ECs and DCs is essential for initiation and perpetuation of the disease. When stimulated by allergens, structural ECs create a specific inflammatory environment that instructs DCs to initiate the immune response. The characteristics of a particular allergen and insults by other environmental triggers are able to modulate the EC-derived inflammatory milieu and the type of immune response that will be generated. Insights into these mechanisms are crucial to develop new therapies that selectively inhibit the natural course of the disease.

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

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