

# Allergens and the airway epithelium response: Gateway to allergic sensitization

Bart N. Lambrecht, MD, PhD,<sup>a,b,c</sup> and Hamida Hammad, PhD<sup>a,b</sup> *Ghent, Belgium, and Rotterdam, The Netherlands*

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1. To identify some of the key mediators in the activation of epithelial cells by allergens.
2. To understand how protease allergens can disrupt the epithelial barrier.

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Allergic sensitization to inhaled antigens is common but poorly understood. Although lung epithelial cells were initially merely regarded as a passive barrier impeding allergen penetrance, we now realize that they recognize allergens through expression of pattern recognition receptors and mount an innate immune response driven by activation of nuclear factor  $\kappa$ B. On allergen recognition, epithelial cells release cytokines, such as IL-1, IL-25, IL-33, thymic stromal lymphopoietin, and GM-CSF, and endogenous danger signals, such as high-mobility group box 1, uric acid, and ATP, that activate the dendritic cell network and other innate immune cells, such as basophils and type 2 innate lymphoid cells. Different allergens stimulate different aspects of

this general scheme, and common environmental risk factors for sensitization, such as cigarette smoke and diesel particle exposure, do so as well. All of this is influenced by genetic polymorphisms affecting epithelial pattern recognition, barrier function, and cytokine production. Therefore, epithelial cells are crucial in determining the outcome of allergen inhalation. (*J Allergy Clin Immunol* 2014;134:499–507.)

**Key words:** Asthma, epithelium, dendritic cells,  $T_H2$

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From <sup>a</sup>the Laboratory of Immunoregulation, VIB Inflammation Research Center, Ghent; <sup>b</sup>the Department of Respiratory Medicine, University Hospital Ghent; and <sup>c</sup>the Department of Pulmonary Medicine, Erasmus MC, Rotterdam.

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Corresponding author: Bart N. Lambrecht, MD, PhD, VIB Inflammation Research Center, Ghent University, Technologiepark 927, 9000 Ghent, Belgium. E-mail: [bart.lambrecht@ugent.be](mailto:bart.lambrecht@ugent.be).

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Allergic asthma is characterized by eosinophilic airway inflammation, mucus overproduction, and bronchial hyperreactivity causing variable airway narrowing. Per definition, known allergens, such as house dust mites (HDMs), animal dander, plant and tree pollen, and fungal spores, trigger the production of IgE by allergen-specific B lymphocytes under the control of IL-4 produced by allergen-specific CD4 T lymphocytes that can also control persistent airway inflammation. Traditionally, therefore, asthma has been seen as a disease of the adaptive immune system, whereby lymphocytes overreact to harmless antigens and mount a type 2 immune response, subsequently causing the activation of

**Abbreviations used**

|                 |                                    |
|-----------------|------------------------------------|
| AAM:            | Alternatively activated macrophage |
| BEC:            | Bronchial epithelial cell          |
| cDC:            | Conventional dendritic cell        |
| DC:             | Dendritic cell                     |
| FCP:            | Fibrinogen cleavage product        |
| HDM:            | House dust mite                    |
| HM-GB1:         | High-mobility group box 1          |
| ILC2:           | Type 2 innate lymphoid cell        |
| moDC:           | Monocyte-derived dendritic cell    |
| NF- $\kappa$ B: | Nuclear factor $\kappa$ B          |
| PAR-2:          | Protease-activated receptor        |
| PRR:            | Pattern recognition receptor       |
| TLR:            | Toll-like receptor                 |
| TSLP:           | Thymic stromal lymphopoietin       |

effector cells, such as mast cells, basophils, and eosinophils. This view has changed considerably over the last years to accommodate the concept that cells of the innate immune system also contribute significantly to disease pathogenesis by recognizing allergens and providing an early warning system through production of cytokines and danger signals.

In this review article we will mainly discuss the innate immune functions of barrier epithelial cells (bronchial epithelial cells [BECs]) of the airways as they respond to inhaled allergens. We will focus specifically on the concept that BECs sense the presence of allergens in inhaled air and subsequently relay this information to airway dendritic cells (DCs), the most proficient antigen-presenting cells of the lung, endowed with the capacity to translate information from epithelial cells and the nature of the allergen to a signal that can be read by T and B lymphocytes. In response to allergen recognition, epithelial cells also orchestrate the early recruitment and activation of type 2 innate lymphoid cells (ILC2s) by using the same activation signals that also activate DCs. Thus we propose that the BEC/DC/ILC2 interaction bridges innate and adaptive immunity at the origin of the allergic sensitization process.

## ACTIVATION OF EPITHELIAL CELLS BY ALLERGENS DEPENDS ON TOLL-LIKE RECEPTORS AND OTHER PATTERN RECOGNITION RECEPTORS

Although immune cells control the many features of asthma, it is unclear why and how they react to allergens. Most organisms that contain allergens, such as pollinating plants, HDMs, cockroaches, and environmental fungi, are not intrinsically pathogenic to the host. However, some proteins or lipids of these organisms or some bacterial contaminants of allergens trigger pattern recognition receptors (PRRs) of the mammalian immune system. One example is HDM, which is known to contain at least 20 allergens that can induce IgE responses in human subjects. In mice development of asthma to HDM strictly depends on Toll-like receptor (TLR) 4 expression on airway epithelial cells.<sup>1-5</sup> This promoting effect of TLR4 ligands on T<sub>H</sub>2 immunity seems counterintuitive at first sight because TLR4 triggering is well known to induce IL-12 production in DCs. IL-12 is a strong polarizing cytokine for T<sub>H</sub>1 immunity in the lungs that suppresses T<sub>H</sub>2 immunity.<sup>6</sup> However, the T<sub>H</sub>2 bias of TLR4 triggering in the lung epithelium has also been noticed with inert proteins in which LPS was a contaminating factor or to which LPS was artificially

added.<sup>7-9</sup> The triggering of TLR4 on epithelial cells by real allergens, such as HDM, is thought to result in part from the Der p 2 allergen, which structurally and functionally resembles the TLR4 and CD14 cosignaling molecule myeloid differentiation protein-2, which is necessary for optimal TLR4 signaling.<sup>10,11</sup> In patients with cat allergy, the dander protein Fel d 1 facilitates TLR4 and TLR2 triggering by facilitating the binding of LPS to TLR4 rather than by acting as an MD-2 mimic. This lipid transfer activity of Fel d 1 is also seen with the dog dander allergen Can f 6, a member of a distinct class of lipocalin allergens.<sup>12</sup>

Although many allergens contain environmental LPS or contain their own microbiota (eg, fecal pellets of HDM) that could act as the agonist for TLRs, it is also possible that TLR4 gets triggered by endogenous danger signals that are released on exposure to allergens. HDM and cockroach allergens lead to the production by epithelial cells of the danger signal high-mobility group box 1 (HM-GB1) protein, a known ligand of TLR4 and of the receptor for advanced glycation end products.<sup>13</sup> Because BECs express a wide range of PRRs, it is likely that TLRs other than TLR4 might be involved in the induction or exacerbation of HDM-induced asthma. Although TLR2 was not involved in the induction of allergic asthma features,<sup>3,14</sup> its triggering on human lung BECs can trigger cytokines, such as GM-CSF and IL-6, and in this way contribute to allergic airway inflammation.<sup>15</sup>

Along the same line, TLR3 triggering in the presence of T<sub>H</sub>2 cytokines strongly enhances thymic stromal lymphopoietin (TSLP) production.<sup>16</sup> Therefore it is likely that the consecutive or simultaneous triggering of TLRs on BECs might facilitate sensitization or favor asthma exacerbations.

Genetic studies have identified risk alleles in the gene locus coding for TLR4 associated with risk of becoming sensitized to several allergens, including HDM and sensitization to laboratory animals.<sup>17,18</sup> However, epidemiologic and experimental studies have highlighted the dose dependency of LPS exposure on either promoting (low dose) or suppressing (high dose) the development of allergic sensitization.<sup>8,19</sup> A high dose of LPS administered simultaneously with allergens can indeed suppress T<sub>H</sub>2 immunity by inducing IL-12 production in DCs or by recruiting myeloid suppressor macrophages.<sup>20,21</sup> Although this might be through direct effects of high-dose LPS on these immune cells, an increase in the threshold for TLR4 triggering (controlled by negative regulators, such as single Ig IL-1 related receptor/TIR8 and A20/TNF- $\alpha$  induced protein 3) on epithelial cells seems to suppress T<sub>H</sub>2 immunity.<sup>22</sup> A reduction of the TLR4 signaling threshold has the opposite effect and promotes allergy. Normally, epithelial cells are hyporesponsive to TLR4 stimulation because they express little TLR4, MD-2, and CD14, all of which are necessary for optimal signaling. However, prior infection history or exposure to environmental pollutants, such as cigarette smoke, increases the surface expression of TLR4 and MD-2, explaining how these environmental factors can promote allergic sensitization, even to harmless protein antigens.<sup>23,24</sup>

Epithelial cells express many different TLRs, and it is clear that many of the effects just described for HDM and TLR4 can also be true for other allergens acting through different TLRs. Recently, the crystal structure of the cockroach allergen Bla g 1 revealed it to be another class of lipid-binding protein that could facilitate TLR ligand binding.<sup>25</sup> Ragweed allergens have been shown to trigger TLRs on various epithelia, thus promoting T<sub>H</sub>2 immunity.<sup>26</sup> Other PRRs might also play a role. Epithelial expression of the C-type lectin receptor Dectin-1 was shown to mediate

recognition of  $\beta$ -glucan motifs in the inhaled fungus *Aspergillus fumigatus* and in several other environmental allergens, such as pollens, HDM, and animal dander.<sup>27,28</sup> Dectin-2 is not expressed by BECs, yet it is crucial for activation of DCs in response to HDM allergen.<sup>29,30</sup>

## PROTEASE ALLERGENS TRIGGER PROTEASE-ACTIVATED RECEPTORS AND TLRs

Some allergens (eg, the HDM allergens Der p 1 and Der p 9, *Aspergillus* species allergens, cockroach allergens, pollen allergen, and the food allergen papain) have proteolytic activity, and it is logical to assume that they function by triggering protease-activated receptors (PARs; eg, PAR-2) on barrier epithelial cells.<sup>31-33</sup> However, in a recent study Millien et al<sup>34</sup> also found that TLR4 plays a crucial role in allergy to fungal proteases, such as those derived from environmental *Aspergillus* species. Colonization by *Aspergillus* species is frequently found in patients with severe asthma and causes allergic bronchopulmonary aspergillosis, a syndrome of eosinophilic pulmonary infiltrates, high IgE production, and central bronchiectasis. Strikingly, the activation of TLR4 by fungal proteases was indirect and required the presence of a serum factor. Fungal proteases cleave the serum factor fibrinogen, thus causing clot formation and releasing fibrinogen cleavage products (FCPs) that can activate TLR4. Thrombin, the classical activator of coagulation, is also able to generate these FCPs from fibrinogen, thus triggering TLR4. The thrombin inhibitor hirudin was able to suppress *Aspergillus* species-driven asthma but, remarkably, also suppressed asthma driven by the model antigen ovalbumin, which is devoid of protease activity. Previous studies in human subjects and mice have demonstrated that allergen challenge is accompanied by plasma extravasation, platelet aggregation, and activation of the coagulation cascade in the lung interstitium and bronchoalveolar compartment, with increases in tissue factor, thrombin, and fibrinogen levels, explaining how these FCPs could be generated with most allergens.<sup>35</sup>

## ALLERGENS CAN DISRUPT EPITHELIAL BARRIER FUNCTION

Protease allergens are proallergenic for other reasons as well. The airway epithelium was traditionally thought to function mainly as a physical barrier and for mucociliary clearance. The integrity of the epithelial barrier depends on apical tight junctions and adherens junctions, which keep BECs together and maintain their apicobasal polarity.<sup>36</sup> Some allergens (eg, Der p 1) contain cysteine proteinase activity that can cleave crucial molecules that make up the epithelial tight junctions (eg, occludin and claudin). In this way protease allergens can increase the accessibility to airway DCs that extend their dendrites just beneath the tight junctions in the intercellular space between epithelial cells.<sup>37</sup> In the airways of asthmatic patients, the epithelia are fragile, and some areas of epithelial basement membrane seem to be denuded of ciliated cells.<sup>36,38</sup> E-cadherin is a major adherens junction component protein, the expression of which is reduced in biopsy specimens of human asthmatic patients as a possible result of epithelial to mesenchymal transition.<sup>39,40</sup> Loss of E-cadherin expression could facilitate allergic sensitization through activation of DCs that receive a tonic inhibitory signal through homotypic E-cadherin interactions between DCs and ECs.<sup>41,42</sup>

Recently, extracts from *Alternaria* species were shown to increase the epithelial permeability of cultured human BECs but only when these cultures were derived from human asthmatic patients and not healthy control subjects.<sup>43</sup> Genome-wide association studies have found associations between genes involved in epithelial integrity and increased risk of atopy and asthma. The proteins encoded by cadherin-related protein 3 (*CDHR3*) and protocadherin 1 (*PCDH1*) are highly expressed in epithelial cells and might control epithelial polarity and integrity and thus facilitate allergic sensitization.<sup>44,45</sup> Loss of epithelial polarity caused by allergen exposure can also lead to release, accessibility, or both of apical cytokine receptors to cytokines or chemokines that are normally only expressed at the basolateral side of the epithelium. In only one example exposure of human BECs to *Phleum pratense* pollen extracts (with low protease activity) led to polarized apical secretion of DC-attractive chemokines, although only in BECs of asthmatic patients, without any effects on epithelial permeability.<sup>46</sup> BECs from asthmatic patients might have pre-existing basal differences, such as a different sensitivity to proteases, and could therefore respond in a stronger and different way to different allergens. Septin-2 was found to regulate apicobasal polarization and prevent disruption of lung epithelial integrity.<sup>47</sup> Whether septin-2 is differentially expressed in asthmatic patients versus healthy control subjects remains to be elucidated. It is striking that IL-13, a prominent effector molecule of  $T_H2$  and ILC2 cells, also disrupts epithelial barrier function. However, IL-13 can also be produced by injured BECs and favors epithelial repair.<sup>48</sup> Therefore IL-13 seems to have a dual role: low levels of IL-13 would favor BEC migration and wound healing, whereas overproduction of IL-13 might facilitate allergic sensitization, perpetuate ongoing inflammation, and lead to airway remodeling.<sup>49</sup>

## ACTIVATED EPITHELIAL CELLS RECRUIT AND ACTIVATE DCs

Triggering of epithelial cells by allergen exposure through PRRs, PARs, and induction of reactive oxygen species often leads to activation of epithelial nuclear factor  $\kappa$ B (NF- $\kappa$ B) signaling.<sup>50</sup> Many of the substances that activate NF- $\kappa$ B in the airway epithelium are also capable of acting as adjuvants to elicit antigen-specific sensitization to concomitantly inhaled protein, thereby circumventing the inherent bias of the lung to promote tolerance to innocuous antigens. Mice lacking key components of the NF- $\kappa$ B pathway or expressing dominant negative NF- $\kappa$ B intermediates that inhibit NF- $\kappa$ B nuclear translocation in the epithelium do not mount allergic inflammation.<sup>51</sup> Conversely, constitutive overexpression of the constitutively active mutant of the inhibitor of NF- $\kappa$ B (I $\kappa$ B) kinase  $\beta$  ([CA]IKK $\beta$ ) that activates NF- $\kappa$ B promotes sensitization to harmless antigens.<sup>52</sup> Epithelial activation leads to production of chemokines, cytokines, and endogenous danger signals that recruit and activate innate immune cells, such as DCs, ILC2s, eosinophils, and basophils. DCs are crucial antigen-presenting cells of the airways that have the potential to take up and present inhaled allergens to CD4 and CD8 T cells when they migrate to the draining mediastinal lymph nodes.<sup>53</sup> Research from several laboratories has shown that DCs play a crucial role in inducing allergic sensitization to inhaled allergens (reviewed by Lambrecht and Hammad<sup>54</sup>). There are several subsets of DCs broadly divided into conventional dendritic cells (cDCs; further consisting of CD103<sup>+</sup> and CD11b<sup>+</sup> subsets),

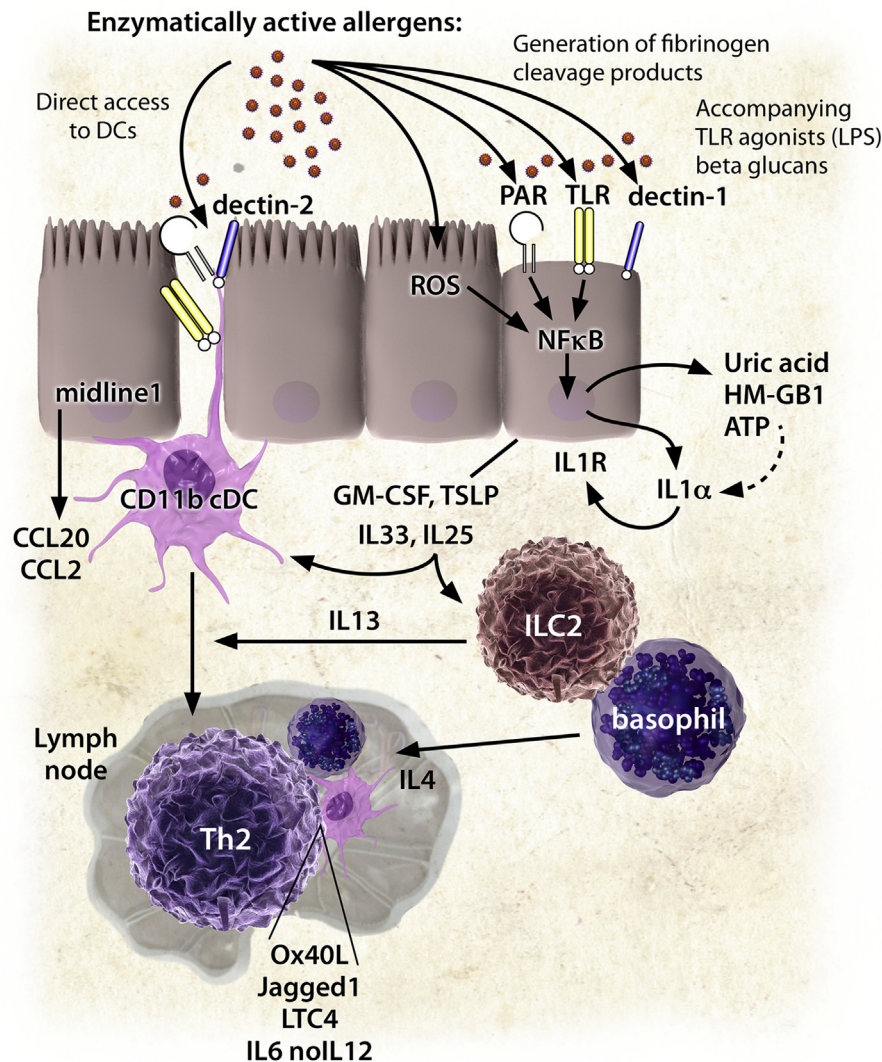


**FIG 1.** Role of BECs on induction of allergic sensitization. Inhaled proteolytic allergens, such as HDMs, can activate different PRRs (TLR, PAR, and Dectin) on BECs and DCs and can cleave epithelial tight junctions, gaining access to the DC network. Some fungal proteases can cleave fibrinogen into FCPs, which can activate epithelial TLR4. Other allergens also induce the production of reactive oxygen species (ROS), which activate DCs or BECs through NF-κB activation. The result of PRR activation and ROS production in BECs is the production of endogenous danger signals, such as uric acid, ATP, and HMGB1, as well as DC activation (IL-1α, GM-CSF, and IL-33). BECs exposed to allergens also induce the recruitment of IL-13-producing ILC2s. All these signals allow DCs to migrate to the T-cell area of the draining lymph nodes, where they interact with naive T cells and induce Th<sub>2</sub> responses. This Th<sub>2</sub> differentiation is influenced by secreted cytokines and the expression of surface molecules, such as OX40 ligand (OX40L), by DCs. Basophils are an important source of IL-4 that further support DC-induced Th<sub>2</sub> differentiation.

plasmacytoid DCs, and monocyte-derived dendritic cells (moDCs).<sup>55-57</sup> Some of these DC subsets induce Th<sub>2</sub> immunity to allergens (CD11b<sup>+</sup> cDCs and moDCs), whereas others, such as plasmacytoid DCs and CD103<sup>+</sup> cDCs, induce tolerance to inhaled antigens.<sup>58-60</sup> The recruitment of lung cDCs and moDCs in response to HDM allergen inhalation strictly depends on TLR4 expression on radioresistant epithelial cells that produce CCL20 and defensins (acting through CCR6) and CCL2 (acting on CCR2).<sup>1,61,62</sup> Production of CCL20 by airway epithelial cells in mice and human subjects depends on the E3 ubiquitin ligase midline-1, which suppresses the activity of a protein

phosphatase-2A.<sup>2</sup> The production of CCL20 by epithelial cells can also be induced through triggering of Dectin-1 and PAR-2 (eg, by cockroach allergens).

Epithelial cells activated by allergens not only recruit DCs to the site of allergen exposure, they also induce DC activation and a propensity of DCs to induce Th<sub>2</sub> immune responses when migrating to the lung. Induction of Th<sub>2</sub> immune responses by DCs in the lungs is incompletely understood but involves induction of OX40 ligand,<sup>63</sup> the notch ligand jagged-1, and other costimulatory molecules on DCs and their production of soluble mediators, such as leukotriene C<sub>4</sub>, cytokines (IL-6, IL-33, and

IL-1 $\beta$ , but not IL-12, which inhibits T<sub>H</sub>2 immunity), and the chemokines CCL17 and CCL22.<sup>29,64</sup> The best way to generate this type of DC activation is through indirect activation of the DCs through stromal or epithelial cells that produce cytokines, such as GM-CSF, TSLP, IL-33, and IL-25.<sup>65-72</sup> In response to HDM inhalation, these cytokines are made simultaneously in a process requiring TLR4 triggering on epithelial cells<sup>1</sup> but also the production of the endogenous danger signal uric acid (Fig 1).<sup>5</sup> TLR and uric acid triggering of the NLRP3 inflammasome are classical triggers of caspase-1 activation and IL-1 $\beta$  secretion. Sensitization to inhaled HDM depends on signaling through the IL-1 receptor, yet the NLRP3 inflammasome caspase-1 or IL-1 $\beta$  was not involved.<sup>4,5</sup> However, in models of skin sensitization to HDM, there appears to be a role for the Nlrp3 inflammasome.<sup>73</sup> In response to allergen exposure and TLR4 stimulation, epithelial cells produce IL-1 $\alpha$  that feeds back in an autocrine manner onto the IL-1 receptor expressed by epithelial cells to further boost production of GM-CSF, IL-33, IL-25, and TSLP and the endogenous danger signal HM-GB1.<sup>4,13</sup> In human BECs IL-1 $\alpha$  also induces the release of GM-CSF and IL-33.

## RELEASE OF ALARMIN IN RESPONSE TO ALLERGEN INHALATION

Alarmins are cytokine-like molecules that are normally sequestered intracellularly yet released on cellular stress or death. Patients with allergic asthma express higher levels of the alarmin IL-33 in the airway mucosa and serum compared with healthy subjects.<sup>74</sup> Recent genome-wide association studies on the genetics of asthma have identified single nucleotide polymorphisms in the gene coding for the IL-33 receptor IL-1RL1 (aka T1/ST2), as well as in the IL-33 locus itself.<sup>75</sup> In mice neutralization of IL-33 blocks the development of lung T<sub>H</sub>2 immunity to a number of allergens, as well as lung-dwelling parasites.<sup>4,66,76</sup> IL-33 has broad effects on both innate (macrophages, DCs, ILC2s, basophils, and eosinophils) and adaptive (T<sub>H</sub>2 cells) immunity and can also act as a transcriptional regulator.<sup>53,66,70,74,77,78</sup>

It is not known how IL-33 is released from epithelial and immune cells and whether IL-33 release results from cell death mechanisms or an active secretion process.<sup>79</sup> IL-33 does not contain a signaling peptide for secretion through the endoplasmic reticulum and Golgi pathway, yet there is a dynamic flux between IL-33 located in the nucleus and the secretory vesicles.<sup>77</sup> During necrosis, IL-33 remains in its active form, whereas under conditions of apoptotic cell death, the executors caspase-3 and caspase-7 cleave IL-33 into an inactive form.<sup>80</sup> In an HDM-driven murine model of asthma, the epithelial repair factor Trefoil factor 2 was shown to induce IL-33 production in airway epithelia, alveolar macrophages, and Fc $\epsilon$ RI<sup>+</sup> inflammatory DCs and thus contributes to induction of T<sub>H</sub>2 immunity in a process requiring the Trefoil factor-2 receptor CXCR4.<sup>76</sup>

Other alarmins, such as HM-GB1, which are typically released in response to necrotic cell death, also seem to be released at an increased rate in allergen-exposed airways, but again, it is unclear whether HM-GB1 is released from dying cells or through an active process.<sup>81,82</sup> Macrophages and DCs have indeed been shown to release HM-GB1 when triggered by PRRs. Along the same lines, we have found that uric acid, a breakdown product of DNA metabolism in dying cells, as well as ATP, which is released from dying cells, is found in increased concentrations in the airways of mice and human subjects with asthma. Again,

it is unclear whether uric acid and ATP release are really the result of increased cell death in the airways because the enzyme generating uric acid hypoxanthine oxidoreductase is also induced by TLR4 triggering.<sup>5,83</sup>

A recent report in *Nature* by Juncadella et al<sup>84</sup> adds a further level of complexity to the function of airway epithelial cells and cell death. Apoptotic epithelial cells are engulfed by viable BECs in a process requiring the small GTPase Rac1.<sup>84</sup> Rac1 is classically involved in the engulfment of large extracellular material by phagocytes and DCs.<sup>85</sup> Deficiency of Rac1 selectively in mouse BECs led to defective uptake of injected apoptotic epithelial cells *in vitro* and *in vivo*. Uptake of apoptotic cells by viable epithelial cells leads to the production of IL-10 and TGF- $\beta$  in epithelial cells, which suppresses the activation of immune cells. Administration of ovalbumin or HDM containing little LPS in mice lacking Rac1 in BECs led to increased development of T<sub>H</sub>2 immunity and the salient features of asthma, whereas this was not the case in wild-type mice. When BECs lacked Rac1, there was an increase in IL-33 alarmin production on low-dose HDM exposure, explaining how sensitization was induced by allergens containing little endotoxin.<sup>84</sup>

## EPITHELIAL CYTOKINES ALSO ACTIVATE ILC2s TO PROMOTE TYPE 2 IMMUNITY

In response to allergen recognition, epithelial cells not only promote T<sub>H</sub>2 immunity through activation of DCs but also do so by activating innate immune cells, such as ILC2s and basophils, which can help DCs to polarize T<sub>H</sub>2 responses in naive T cells.<sup>56</sup> The fact that eosinophil-rich responses can also be induced in mice lacking T and B cells suggested a potential role for ILCs during allergic immune responses.<sup>86</sup> Murine ILC2s express CD127, Sca-1, T1/ST2, and IL17RB, the receptor for IL-25. When activated by epithelial cytokines, such as IL-25 or IL-33, the ILC2s can control some of the features of asthma, such as AHR, goblet cell hyperplasia, and eosinophilia,<sup>87-94</sup> through production of IL-5, IL-9 and IL-13. In mice ILC2s derive from committed T1/ST2<sup>+</sup> pre-ILC2s that develop from common lymphoid progenitors in the bone marrow under the influence of IL-33, IL-25, or both but not TSLP. Strikingly, T1/ST2<sup>+</sup> ILC-2 and pre-ILC2s can be identified in Gata3 reporter mice.<sup>95,96</sup> The master transcription factors for ILC2 development are retinoic acid-related orphan receptor  $\alpha$  and GATA3.<sup>97-99</sup> A single nucleotide polymorphism in the gene encoding retinoic acid-related orphan receptor  $\alpha$  was discovered to be associated also with human asthma,<sup>75</sup> but it is unclear whether this would lead to more or less ILC2 development in the setting of asthma or would mainly affect epithelial biology.

Several allergens (HDM, *Alternaria* species, and papain) have been shown to induce ILC2 recruitment, proliferation, or both in the lungs. The precise signals involved in recruitment of ILC2s are currently unknown, but mRNA expression data suggest that the same chemokine receptors that attract T<sub>H</sub>2 cells to the lungs (CCR4, CCR8, and chemoattractant receptor-homologous molecule expressed on T<sub>H</sub>2 cells) might be involved, explaining why ILC2 accumulation depends on signal transducer and activator of transcription 6.<sup>100</sup> Conversely, the signals that dampen ILC2 recruitment are only now being recognized. Lipoxin A<sub>4</sub> is an epithelial resolvin that suppresses ILC2 accumulation.<sup>101</sup>

ILCs also contribute to the allergenicity of chitin.<sup>102</sup> Chitin is a polysaccharide found in the exoskeletons of parasites and arthropods and the hyphal cell walls of fungi. When inhaled, it

immediately causes eosinophil-rich airway inflammation and accumulation of alternatively activated macrophages (AAMs) that typically produce arginase and express chitinases under the influence of IL-4, IL-13, or both. By using IL-13<sup>smart</sup> mice, which report on IL-13 protein levels without affecting endogenous IL-13 levels, and IL-5<sup>RFP</sup> reporter mice, the source of IL-5 and IL-13 cytokines was shown to be exclusively ILC2s. Whereas the eosinophilia was dependent on ILC2-derived IL-5 and IL-13, the AAM phenotype was dependent exclusively on ILC2-derived IL-13. With use of dual-photon imaging to image deep in the lung tissue, ILC2s were found in close vicinity to blood vessels, explaining how IL-5 could also be released in the bloodstream to affect the bone marrow output of eosinophils. The authors cleverly made use of Cre-expressing mice in which ILCs are killed as soon as they commit to IL-5 or IL-13 cytokine production to delete ILC2s in mice with a functional immune system. ILC deletion led to reductions in early eosinophilia and AAM induction comparable with levels seen in *Rag*<sup>-/-</sup>*γc*<sup>-/-</sup> mice. Unexpectedly, ILC2 deletion also led to increased production of TNF-α, IL-1β, and IL-23 and increased production of IL-17 by innate γ-δ T cells, causing increased airway neutrophilia. These suppressive effects of ILC2s on neutrophil and γ-δ T-cell activation were not due to defective IL-5 production, IL-13 production, or both but rather to an unidentified mechanism of suppression. ILC2s express receptors for TSLP, IL-33, and IL-25. Chitin inhalation induced these pro-T<sub>H</sub>2 cytokines in the lung, but the combined absence of all 3 cytokine receptors did not affect ILC2 numbers, merely their activation status and production of IL-5/IL-13.

## RISK FACTORS FOR ALLERGIC SENSITIZATION AND EPITHELIAL RESPONSES

Environmental risk factors, such as viral respiratory tract infections, cigarette smoke exposure, fine particulate air matter exposure, and toxic gases, might facilitate the development of allergy through some of the mechanisms we have just described. For example, infection with respiratory syncytial virus or exposure to cigarette smoke increases the surface expression of TLR4 and MD-2, thus decreasing the threshold for allergen recognition.<sup>23,24</sup> This increased sensitivity of BECs to TLR4 ligands might help explain how prior infections or pollutants might act as predisposing factors for allergic sensitization. Certain viral infections (influenza virus and paramyxoviridae), air pollution with NO<sub>2</sub> or fine particulate matter, and cigarette smoke can also lead to reactive oxygen species production, NF-κB activation, and epithelial cytokine production of IL-1, IL-33, IL-25, TSLP, HM-GB1, uric acid, and ATP, thus activating DCs and ILC2s.<sup>103-108</sup> Strikingly, at the same time, many of these stimuli also disrupt epithelial barrier function. RSV infection of BECs induced increased permeability associated with actin remodeling and the disruption of intercellular junctions.<sup>109</sup> RSV infection sensed by BEC retinoic acid-inducible gene 1 and TLR3 was recently shown to lead to TSLP production and to favor allergic responses and asthma.<sup>110</sup> By comparing RSV-exposed BECs from asthmatic patients and healthy subjects, Lee and Ziegler<sup>111</sup> found that only cultures from asthmatic patients showed increased TSLP production. Other environmental triggers, such as diesel exhaust particles, induce TSLP production in BECs, leading to upregulation of the pro-T<sub>H</sub>2 proteins OX40 ligand and Jagged-1 on DCs and to altered airway responses.<sup>68</sup> Therefore environmental triggers might act

together or in a sequential manner to promote allergic sensitization through the release mediators of T<sub>H</sub>2 immunity.<sup>112,113</sup>

## Conclusions

Careful study of the process of sensitization to inhaled allergens, as well as genetic studies on the risk of allergic sensitization, have uncovered the molecular pathways that lead to activation of innate and adaptive immune responses. Whereas epithelial cells were initially merely regarded as a passive barrier impeding allergen penetrance, we now realize that epithelial cells recognize allergens and mount an innate immune response composed of cytokines, alarmins, and endogenous danger signals that activate the DC network and other innate immune cells. Therefore epithelial cells are crucial in determining the outcome of allergen inhalation. In the future, we need to understand more how these pathways, mainly those studied in mice, operate in human subjects and how environmental risk factors and genetic polymorphisms affect the crosstalk between epithelial cells, DCs, and innate immune cells.

### What do we know?

- We now have a clear picture of how T<sub>H</sub>2 immunity to inhaled antigen develops.
- Epithelial cells get activated to produce chemokines and cytokines that recruit and activate DCs.
- DCs migrate to the lymph nodes and collaborate with innate immune cells to induce T<sub>H</sub>2 immunity.
- Many allergens either activate epithelial cells directly, or lead to the formation of endogenous molecules that activate epithelial cells and DCs.

### What is still unknown?

- We do not know if this model derived from murine studies is identical in humans.
- We do not know if this model is true for all allergens.
- We are only beginning to understand how genetics and environment influence the epithelial-DC crosstalk that leads to allergy.

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