

REVIEW

Airway responses towards allergens – from the airway epithelium to T cells

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Summary

The prevalence of allergic diseases such as allergic rhinitis is increasing, affecting up to 30% of the human population worldwide. Allergic sensitization arises from complex interactions between environmental exposures and genetic susceptibility, resulting in inflammatory T helper 2 (Th2) cell-derived immune responses towards environmental allergens. Emerging evidence now suggests that an epithelial dysfunction, coupled with inherent properties of environmental allergens, can be responsible for the inflammatory responses towards allergens. Several epithelial-derived cytokines, such as thymic stromal lymphopoietin (TSLP), IL-25 and IL-33, influence tissue-resident dendritic cells (DCs) as well as Th2 effector cells. Exposure to environmental allergens does not elicit Th2 inflammatory responses or any clinical symptoms in nonatopic individuals, and recent findings suggest that a nondamaged, healthy epithelium lowers the DCs' ability to induce inflammatory T-cell responses towards allergens. The purpose of this review was to summarize the current knowledge on which signals from the airway epithelium, from first contact with inhaled allergens all the way to the ensuing Th2-cell responses, influence the pathology of allergic diseases.

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Introduction

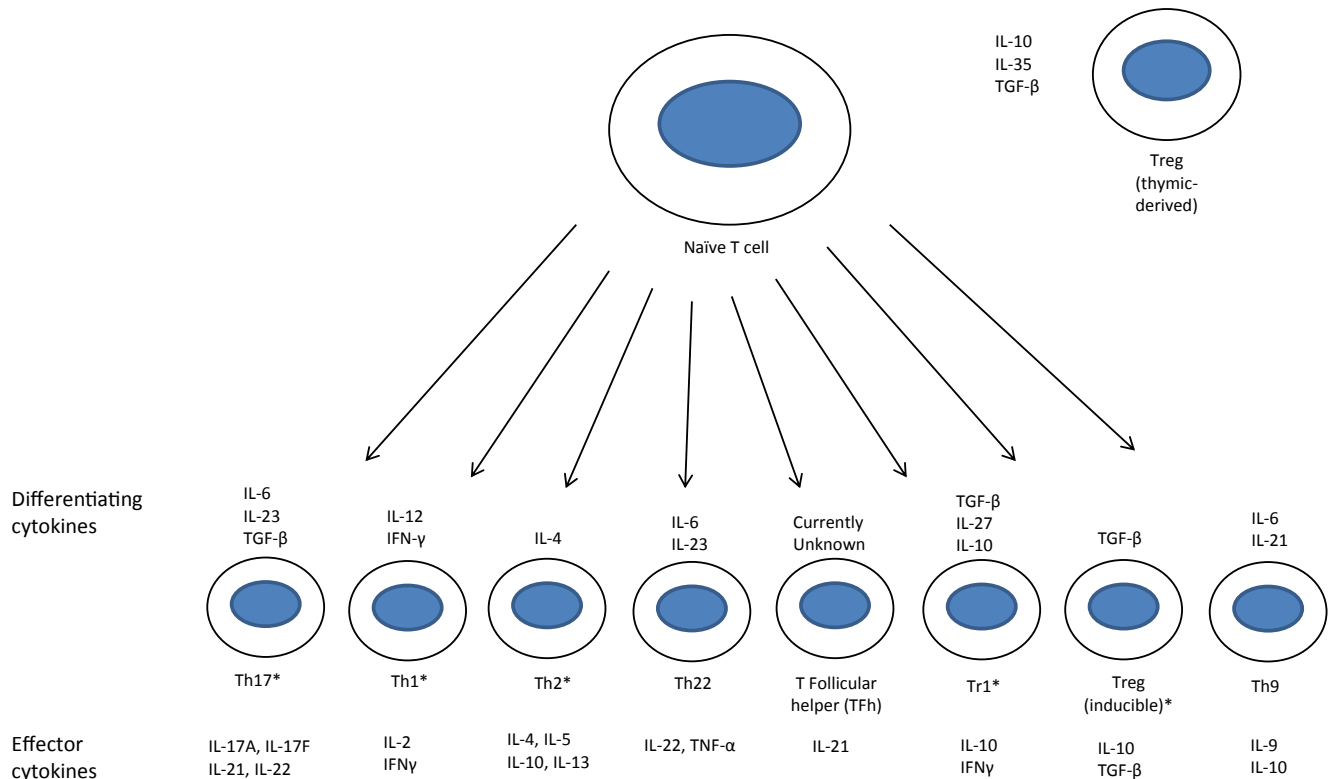
When exposed to foreign pathogens, our adaptive immune system responds in a variety of different ways, depending on the signalling from the innate immune system in response to specific pathogen-associated molecular patterns (PAMPs). Allergic diseases affect around 30% of the world's population [1], and the sensitization arises from complex interactions between environmental exposures and genetic susceptibility, resulting in a Th2-skewed response [2]. Since the initial description of Th1 and Th2 cells, an expanding array of cytokines and chemokines has been described that gives rise to additional pro-inflammatory and suppressive (or regulatory) T-cell subsets such as Treg, Tr1, Th17, Th9, and Th22 cells (Fig. 1), and these T-cell subsets may play a role in asthma and allergy [3].

The airways contain a layer of airway epithelial cells (AECs) that interact with inhaled allergens and pathogens and are able to influence the activation of the ensuing immune responses by influencing tissue-resident or circulating antigen-presenting cells (APCs), such as the dendritic cell (DC) [4, 5]. In the airways, the

immune system maintains a balance between protective responses towards pathogens and the ability to maintain homeostasis in response to harmless foreign antigens, and emerging theories view allergy and asthma as partially epithelial disorders where altered interactions between the epithelium and DCs are a key factor in allergic sensitization [6–8].

Recent discoveries have led to increased understanding of the interplay between AECs and innate immune cells, and the airway epithelium has been shown to direct the activities of DCs and, thereby, affect CD4⁺ T-cell activation and polarization after encounters with inhaled allergens [9]. In their frontline position, the AECs express a multitude of receptors such as protease-activated receptors (PAR), scavenger receptors (SRs) [10–13], and pattern recognition receptors (PRRs) [14–16]. These receptors allow AECs to interact and respond to contact with specific components of allergens with the release of pro-inflammatory and anti-inflammatory cytokines and chemokines.

Tolerance to self-antigens and harmless environmental antigens is maintained, at least in part, by means of active suppression by Treg cells, and DCs play an



* Primary subsets involved in mucosal responses

Fig. 1. The currently defined T helper cell subsets. CD4⁺ naïve T cells can differentiate into a variety of unique effector subsets determined in part by the cytokine milieu that is present when the antigen-presenting cells deliver an antigen. Th cells have been categorized by different properties (such as Tregs) but most often through their secretion of distinct effector molecules of which they exert their functions. New Th subsets are continuously being discovered, and those that have been described to date are Th1, Th2, Th9, Th17, Th22, Tr1, Treg (inducible and thymically derived), and T follicular helper cells.

important role in the initiation of regulatory T-cell responses [17]. DCs with low expression of co-stimulatory molecules induce suboptimal T-cell priming, leading to deletion of T cells [18, 19] or the induction of tolerogenic regulatory T cells that can block or functionally suppress other inflammatory T cells [20–22]. Regulatory T-cell subsets have been reviewed elsewhere [23–25] but can for the purpose of this review be divided into naturally occurring thymically derived CD4⁺ CD25^{hi} (natural) Tregs, characterized by the expression of the transcription factor fork-head box protein 3 (FoxP3), inducible FoxP3⁺ Treg cells that are peripherally induced by antigenic stimulation and triggering of the aryl hydrocarbon receptor in the presence of TGF-β in an IL-10-rich environment, and Tr1 cells that are induced in the absence of TGF-β [26] (see Fig 1). IL-10 appears to be a key cytokine in the development of regulatory T-cell responses in allergic disorders; however, the specific mechanisms controlling IL-10 production from these cell types and whether the balance between IL-10 and pro-inflammatory Th2 cytokines is altered in atopic patients are still unclear.

By describing how epithelial cells, acting as the first layer of defence against allergens, can interact with innate cell populations in its microenvironment, acting as a second layer of defence, that further influence ensuring inflammatory Th2-cell responses, this review provides up-to-date information on the current knowledge of the airway epithelium influence on allergic diseases.

The airway epithelium

The airway epithelium as a physiological barrier

The human lung is exposed daily to 10 000 L of inhaled air containing potentially harmful physical, chemical, and biological agents. The AECs, when functioning normally, form an efficient physical barrier and is the first line of defence against inhaled pathogens and other harmful material [27]. The integrity of the epithelial barrier is maintained largely by the apically located protein complex; tight junctions (TJs) between adjacent epithelial cells regulate transepithelial diffu-

sion, intercellular communication, and transport between adjacent cells [28, 29]. Other defence mechanisms include a protective surfactant film layer [30], the muco-ciliary escalator which transports matter back up the airways [31], and a resident population of macrophages, mast cells, and DCs in the airways [32]. Within the healthy airway epithelium, there are also a constitutive small number of migratory cells comprising T cells, NK cells, and granulocytes [33].

The airways as an immunological barrier

The AECs are equipped with a variety of specialized PRRs that recognize specific PAMPs and DAMPs [34, 35]. In a nonsterile environment as the airways, it is essential that the airway cells discriminate between different pathogenic and nonpathogenic signals to uphold homeostasis or mount an appropriate immune response. One of the suggested methods of doing so is by graded responses, where the epithelium modulates and instructs local immune responses when a certain activation threshold of receptor signalling is reached [36]. Before this threshold is reached, epithelial interactions with DCs would potentially prevent overt Th2-cell responses. Using an *in vitro* co-culture model, we have shown that direct contact between polarized epithelial cells and DCs has a regulatory effect on DC phenotype and cytokine production, leading to attenuated T-cell responses [37, 38]. Similar effects of polarized epithelial cells of the gastric and intestinal mucosa are mainly mediated via the release of soluble mediators maintaining intestinal homeostasis [39]. This so-called conditional DC imprinting [40] suggests that epithelial cells modulate local DC differentiation and that allergic sensitization may be due to a defective innate immune response of epithelial cells.

In patients with asthma, there is an ongoing inflammation of the airways affecting epithelial cells' ability to polarize. *In vitro* studies with AECs from asthmatics showed that these cells fail to polarize to the same extent as AECs taken from healthy individuals [6, 41]. This might be associated with the expression of E-cadherin, a major component of the TJ complex, which is reduced in biopsies from patients with asthma (discussed below) [42–44]. The loss of epithelial integrity and subsequent breach of barrier function may also be due to proteolytic properties of the allergens themselves. This has been shown for house dust mite (HDM) allergens [45], cockroach [46], and certain pollens [47], fungi [48], and mould [49]. Because healthy individuals are exposed to the same allergens, susceptibility towards overt Th2 reactions or/and epithelial dysfunction may be caused by other factors as well. Breakdown of the epithelium can also be caused by exposure to environmental pollutants such as cigarette smoke [50]

and diesel particles [51] and by respiratory infection with RNA viruses [52], or by administration of endogenous Th2 cytokines IL-4 and IL-13 *in vitro* [53, 54]. Together with other recent findings, such as dysfunctional mutations in the filaggrin gene being linked to atopic dermatitis and peanut allergy [55], this has led to epithelial disruption, or dysregulation, being viewed as a key element in allergic diseases [8].

Dendritic cells in the airways

DCs are highly specialized APCs that are frequently located in close proximity to the outer surfaces [56]. DCs in the airways form a dense network and perform a unique sentinel function in airway immune responses in that they recognize inhaled antigens through the expression of various extracellular and intracellular PRRs such as Toll-like receptors (TLRs), nucleotide-binding oligomerization domain protein receptors (NOD-like), and lectin receptors that recognize carbohydrate motifs [57]. DCs are the principal cells involved in directing T-cell responses and differentiation [14]. Upon activation, DCs upregulate expression of co-stimulatory receptors (such as CD80 and CD86) and the mature DCs migrate to local draining lymph nodes where they present antigens and interact with T cells [58].

However, harmless antigens such as allergens do not possess any PAMPs or DAMPs and will fail to mature DCs or induce suboptimal maturation, which induces tolerance [18, 59, 60]. In addition, the delicate balance between DC activation and inactivation also includes which PRR is activated and the strength of the particular activation, whether other PRRs are activated simultaneously, duration of exposure, and additional cytokine signalling from other cells in the microenvironment in which the DCs are located [61].

Airway dendritic cell subsets

DCs have been identified throughout the epithelial mucosa of the airways, from the nose down to the alveolar interstitium [57, 62]. Airway DCs were originally described in mice as a single population of dendritic-shaped cells expressing CD11c and MHC-II [63], but are currently regarded to be at least five different murine subsets in the lungs [64]. In humans, the knowledge regarding lung DC subsets is not as complete. For the purpose of this review, DCs will be broadly divided into 2 major subgroups: the CD11c+ myeloid dendritic cells (mDCs; also referred to as 'conventional' DCs) and the CD11c- plasmacytoid DCs (pDCs) [57]. These two major DC subsets constitute only a minute fraction of circulating mononuclear cells, with around 0.75% for mDCs and 0.3% for pDCs [65, 66]. There is convincing evidence, mainly from animal models, that mDCs and

possibly also pDCs are involved in the pathogenesis of allergen-induced rhinitis. CD11c⁺ mDCs produce IL-12 and are potent inducers of Th1 cytokine secretion [67] but appear to be responsible for the development of secondary immune responses towards inhaled allergens as well [68–70]. pDCs on the other hand do not secrete IL-12. They appear instead to play a role in providing tolerance to inhaled allergens [67, 71, 72] and have also been found to induce the production of the tolerogenic cytokine IL-10 from T cells, even when initiating Th1 responses [73–75]. In allergic asthmatics, mature DCs and immature mDCs, and possibly also pDCs, accumulate rapidly into the airways after allergen challenge. Simultaneously, circulating CD11c⁺ DCs are reduced up to 24 h after allergen inhalation, which indicates that these cells are recruited from the bloodstream in response to allergen challenge [76–78].

Group 2 innate lymphoid cells

Recent discoveries have identified another population of innate cells associated with Th2 responses and allergy and will therefore be briefly discussed. Group 2 innate lymphoid cells (ILC2s) have morphological similarities to lymphocytes, but they do not express T-cell or B-cell antigen receptors and lack cell surface markers associated with other cell lineages of the immune system. ILCs have been categorized into three groups on the basis of their ability to produce cytokines associated with Th1, Th2, or Th17 cells [79]. ILC2 was first identified in mice in a study by Fort and colleagues where they found an induced production of IL-5 and IL-13 in mice lacking B cells and T cells after the administration of Th2-related cytokine IL-25 [80]. In the following years, other groups found that IL-5 and IL-13 were produced by non-B-non-T cells after the administration of IL-25 in helminth- or parasite-infected mice [81, 82]. In 2010, three independent groups identified and further characterized type 2 cytokine-producing ILC2s as cells that produce IL-5 and IL-13 in response to IL-25 and IL-33 [83–85]. In the different studies characterizing ILC2s, different subpopulations were identified in terms of phenotypes and tissue distribution. These subpopulations have been classified as 'natural helper cells', 'innate helper 2 cells', and 'nuocytes'; however, it has been agreed that these cells should all be referred to as 'ILC2 cells' [79, 86, 87]. In the lung, ILC2s are a rare cell population. Under noninflammatory conditions in mice, lung ILC2s only represent 0.25–1% of total live cells [88]. Nonetheless, ILC2s appear to have a critical role in lung inflammation in response to papain and HDM allergen, suggesting that ILC2 might mediate allergic inflammation in response to protease-containing allergens [89]. In human studies, nasal polyps from patients with chronic rhinosinusitis show enrichment

for ILC2 [90], and lesions from patients with atopic dermatitis also show enrichment for ILC2 cells, suggesting their involvement in allergic responses in the skin as well [91].

In murine models of asthma, ILC2s have been found to represent a large proportion of the total IL-5- and IL-13-producing cells in the lungs after allergen exposure [92]. ILC2s may also be involved in the activation of adaptive immune responses as at least some ILC2 cells are reported to express MHC class II [84]. This indicates that ILC2s may play an important part in both early allergic responses through the induction of Th2 inflammation and maintaining the inflammation by secreting high levels of IL-5 and IL-13. ILC2s have also been found to increase in the peripheral blood after nasal allergen challenge, suggesting that they can be used as a biomarker for allergic inflammatory responses (MH Shamji, personal communication). That ILC2 plays an important role in inflammatory Th2 responses is becoming more evident, but more research is still needed to fully elucidate their role and whether they can be a new viable target for treatment in allergic and asthmatic responses.

Local responses in allergic sensitization

Several components of many allergens have the potential to directly affect the epithelial barrier and establish a Th2-skewed microenvironment. It has been hypothesized that epithelial cells' ability to trigger different responses in different tissues could be facilitated by tissue-specific activation pathways [93]. Viral infections of the airway epithelium or exposure to cigarette smoke upregulates the expression of TLR4, thereby increasing the epithelium's responsiveness for endotoxin activation [94, 95]. Cigarette smoke also induces a significant increase in the epithelium's ability to take up allergen, which further potentiates allergic responses [96]. Interestingly, specific activation of TLR4 on hematopoietic cells has been found to be an important step in triggering DC migration to the airways in mice [34]. Other studies in animal models revealed that some allergens, such as HDM and frass (cockroach faeces), directly induce DC maturation and promote the production of inflammatory cytokines [97–99], and this may be potentiated by TLR4 signalling through trace LPS contaminations found in some allergen preparations [100–102]. Conversely, high-dose LPS has been associated with a protective effect against allergy and the development of atopic disease in accordance with the hygiene hypothesis, at least among populations with a certain genotype [103].

The homing properties of DCs are mainly regulated by the chemokine receptors they express. CCL20 is an important chemokine for the recruitment of CCR6-expressing

immature DCs to several tissues for subsequent antigen sampling [104, 105]. Human AECs secrete CCL20 after exposure to HDM, and this secretion is mediated through AEC recognition of distinct b-D-glucan moieties present in HDM allergen [106]. Similarly, stimulation with Der p 1, the main allergen in HDM, induced increased CCL20 production from AECs in HDM-sensitized patients with asthma, leading to increased migration of Langerhans cells, a mDC subpopulation, that reside at mucosal surfaces [105]. In addition, activation of PAR-2 on AECs by allergen-derived proteases induces both CCL20 and GM-CSF in the airways, which increases recruitment and differentiation of mDC populations in the lungs [107].

Several known allergens are proteases and activate PARs on airway DCs [108–110]; however, the ability to directly influence DCs is not a general feature of all allergens. Allergens with protease activity have been found to trigger chemokine responses from AECs independent of PAR receptors in studies with human alveolar cell line A549 [111, 112]; however, allergens without any protease activity such as ovalbumin also possess allergenic activity. Prior studies have linked TLR4 on AECs to both proteinase-dependent and proteinase-independent allergic responses in mice [34, 100], and a recent study by Millien and colleagues in mice found that TLR4 is activated by proteases in the airways to initiate allergic airway disease immunity. These immune responses were induced by protease cleavage of the clotting protein fibrinogen, yielding fibrinogen cleavage products that acted as TLR4 ligands on AECs and macrophages, suggesting a potential link between allergic immune responses and fibrinogen [113].

Several groups have further reported that proteases and lipid components of pollen extracts have the potential to interfere with the epithelial barrier [47, 114]. However, these studies have used epithelial cell lines consisting of undifferentiated, although polarized, cell cultures. In recent studies with differentiated primary epithelial cells at an air–liquid interface, pollen extracts were not able to disrupt the epithelial barrier [115]. With a differentiated cell culture including mucus-secreting cells, the authors theorize that this allows for secreted components such as protease inhibitors to inactivate environmental proteases. Taken together, protease activity does not appear to be a general feature of allergens but appears to be an important pathway for some allergens to activate both epithelial cells and DCs.

Allergens commonly affect innate immune cells indirectly, triggering release of chemokines and cytokines from the AECs, which in turn can recruit neutrophils, eosinophils, mast cells, DCs, and lymphocytes, as well as modulate their responses [104, 105, 116]. Moreover, airway epithelium from allergic or asthmatic individuals reacts differently towards allergen stimulation compared

with AECs from healthy individuals. Primary bronchial epithelial cell cultures from asthmatic donors secrete increased levels of IL-1, GM-CSF, and TNF- α compared with healthy controls [117]. Der p 1 stimulation of AECs from allergic asthmatics, but not from healthy controls, increases the migratory ability of DC precursors [105]. Increased migration of myeloid Langerhans cells and other CD1a⁺ DCs has also been observed in nasal epithelia after grass pollen stimulation in patients with severe asthma [115] and in patients with allergic rhinitis [118–120]. Finally, transcriptomic analyses have revealed that there are differences in number of modified epithelial transcripts between healthy individuals and birch pollen-allergic individuals after birch pollen exposure [121]. Healthy individuals modified a larger number of transcripts, thus raising a strong epithelial response after intranasal allergen challenge, whereas allergic patients had a reduced response, suggesting that allergies could also be due to hyporeactive responses on the epithelial cell level [121, 122].

Allergen uptake by and migration of dendritic cells in the airways

It is unclear whether mucosal DCs mainly sample allergens across the epithelial layer or whether the allergens are mainly transported by the AECs and delivered to DCs situated in the submucosa. Studies on gut epithelial cell lines *in vitro* showed that DCs extend their dendrites across the epithelial barrier by means of the inducible expression of the TJ proteins occludin, claudin-1, and zonula occludens 1 [40], and similar results have been found in studies carried out on biopsies from the nasal epithelium [123]. Interestingly, this process was only seen in allergic individuals, and not in healthy subjects. Similarly, birch pollen was found to be actively transported across the nasal epithelium in only allergic patients [121, 122]. These data suggest inherent differences in epithelial uptake of allergens between healthy and allergic individuals, although more studies are clearly required to fully characterize the mechanisms in play.

Allergen challenge induces expression of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) by AECs, leading to an enhanced production of CCL20, responsible for attracting immature CCR6⁺ DCs and Th2 cells to the airways [124]. Upon allergen uptake, the CCR6 expression is replaced by CCR7 expression, which helps guide DCs to encounter T cells in the lymph nodes via the chemokines CCL19 and CCL21 expressed in the T-cell zones of the lymph nodes [125]. In a mouse model of allergic conjunctivitis, enhanced CCR7 expression was directly correlated with increased mast cell and eosinophil infiltration to the airways and accompanied by substantially increased levels of Th2 cytokines and IgE

[126]. However, CCR7 expression has also been shown to be important in the induction of tolerance towards HDM-induced allergic asthma [127], suggesting that both processes depend on DC migration to and encounter with T cells in lymph nodes.

Dendritic cell interactions with airway epithelial cells

It is generally accepted that healthy epithelial environments by default exert a suppressive effect on DCs, thereby preventing unwanted immune activation, and this has been supported by various *in vitro* studies. Co-culture studies of monocyte-derived DCs with the Caco-2 intestinal epithelial monolayers showed that epithelial-imprinted DCs reduced their expression of MHC class II, CD86, and CD80 and inflammatory cytokines, resulting in poor T-cell stimulatory capacity [128] and also drive Treg development [129]. As a result of increased TGF- β production, co-cultured DCs also became less sensitive to stimulation via TLR2, TLR4, and TLR6 [128]. Mayer and colleagues obtained similar results using the human bronchial epithelial cell line BEAS-2B, where epithelial cell-conditioned DCs, macrophages, and monocytes had decreased secretion of pro-inflammatory cytokines in combination with increased expression of IL-10 and arginase-1 [130]. This was coupled with an increased expression of the co-inhibitory membrane protein PD-L1 (CD274) on DCs, which also hampered subsequent T-cell proliferation [130]. Focusing on direct contact between the epithelium and DCs, we found that AEC-imprinted DCs express higher levels of co-inhibitory receptors PD-L1 and ILT3 (CD85k) and secrete decreased levels of the chemokines eotaxin and monocyte chemoattractant protein (MCP-1) after LPS stimulation. We also investigated AECs' effect on DCs without direct contact and the phenotypical changes were still present, whereas the decreased cytokine responses depended on direct contact with the AECs [38]. Taken together, these results clearly suggest that the airway epithelium influences DCs and subsequent T-cell responses through mechanisms requiring direct contact between AECs and DCs as well as through secreted AEC cytokines.

Surface molecules modulating DC function

Little is known about how direct contact between AECs and neighbouring APCs influences immune reactions, in terms of which receptors are involved and how they exert their function. As discussed above, DCs can interact with epithelial cells by anchoring themselves to the epithelium to sample antigens across the epithelial layer. This is carried out through the expression of adhesion molecules that not only anchor them strongly in the epithelium, but possibly also have an effect on their function.

E-cadherin, ICAM, Inducible T-cell co-stimulator ligand (ICOSL), and CD200

Adhesion mediated by TJ complex component E-cadherin, mentioned earlier under steady-state conditions, triggers an alternative form of DC maturation, whereas the disruption of these interactions induces the typical features of DC maturation [131]. E-cadherin-induced maturation of DCs does not induce release of immunostimulatory cytokines and has been found to induce a more tolerogenic DC profile, which enables the DCs to induce regulatory T cells [131–133]. Epithelial damage induced by the pro-inflammatory cytokine TNF- α has been found to decrease expression of E-cadherin in AECs [134] and disrupt the induction of tolerance. Loss of E-cadherin in cultured AECs has also been found to enhance production of inflammatory Th2 cytokines such as thymic stromal lymphopoietin (TSLP), an important cytokine involved in the induction of inflammatory Th2 responses (discussed below) [135]. Similarly, AECs' expression of intercellular adhesion molecule (ICAM) may also influence airway immune responses. ICAM binds to leucocyte function-associated molecule-1 (LFA-1) expressed by immature DCs [136], and ICAM expression on AECs has been found to be significantly higher in patients with allergic rhinitis and asthma [137] and venom allergies [138]. Treatment against venom allergy by either venom immunotherapy or corticoid treatment significantly reduces expression of ICAM-1 on AECs and in serum, respectively, suggesting that ICAM-1 overexpression is linked to allergic symptoms [138, 139]. Interestingly, ICAM is also expressed by DCs, and the interaction between ICAM and LFA-1 on DC and T cells, respectively, has been shown to induce Th1 responses [140], whereas blocking the ICAM-1/LFA-1 interaction instead promotes production of Th2 cytokines in contrast to the findings above regarding ICAM expression on AECs [141]. Whether interaction between ICAM on AECs and LFA-1 on DCs has similar functional consequences or whether AEC directly interacts with T cells through these surface molecules remains to be determined. However, co-culture studies between epithelial cells and T cells have confirmed that epithelial cells can function as APCs for activated T cells [142–144]. In line with this, interaction between ICOSL on airway intestinal and renal epithelial cells and ICOS on activated T cells enhances T-cell proliferation and cytokine secretion, especially IL-10 [145]. T cells co-cultured with intestinal and renal epithelial cells have a suppressive function, suggesting that regulatory T cells are induced [142, 143], and in murine airways, DCs exposed to allergens induce regulatory T cells that are dependent on ICOS/ICOSL interactions to exert their regulatory function [146, 147]. Another example of DC–AEC interactions in the maintenance of

immunological homeostasis within the lungs is CD200 and its receptor, CD200R [148, 149]. CD200 is expressed on AECs [150], whereas CD200R is expressed almost exclusively by myeloid cells, including macrophages, DCs, neutrophils, basophils, and mast cells [150–153]. Binding of CD200 to CD200R imparts a unidirectional negative signal to CD200R-bearing cells [150, 153, 154], and mice deficient in CD200 show increased activity of alveolar macrophages, leading to death or delayed resolution of airway inflammation [149]. Additionally, CD200–CD200R signalling has been linked to induction of regulatory T cells [154], possibly through the induction of the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO) in plasmacytoid DCs [155]. These observations strongly suggest that various molecular interactions are involved in the regulation of epithelial immune homeostasis even though further research is needed in this expanding area of investigation.

AEC cytokines modulating DC function

Modulation of immune responses by AEC-derived cytokines has been investigated far more thoroughly to date than the effect of AEC surface molecules. Besides secretion of chemokines responsible for recruiting DCs to the epithelium, the AEC-secreted cytokines influence DC maturation and subsequent T-cell polarization. Although TSLP, IL-33, and IL-25 appear to be the main epithelial-derived cytokines that play a major role in allergic Th2 reactions, there are several other cytokines produced by the airway epithelium that are associated with allergic and asthmatic responses, such as GM-CSF, IL-1, IL-6, and TNF- α [156–165]. TSLP in particular has received more attention and appears to be a key AEC-derived molecule at the AEC–DC interface in the initiation of allergic inflammation.

TSLP signalling

TSLP is a 140 amino acid IL-7-like 4-helix-bundle cytokine that is part of the IL-2 cytokine family, comprising IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, IL-21, and TSLP [166]. TSLP was initially identified and characterized as a novel cytokine produced by murine stromal cells in the thymus [167, 168] but was later found to be predominantly produced by epithelial cells of the skin (keratinocytes), lung, and intestine [163, 169]. Epithelial cells can be induced to produce increased levels of TSLP after exposure to viral, bacterial, or parasitic pathogens as well as by activation of Toll-like receptors [170, 171]. In addition, exposure to pro-inflammatory cytokines such as IL-1 β and TNF- α , or to Th2-cell-associated cytokines IL-4 and IL-13, alone or in combination, has also been shown to induce the expression of

TSLP [171–173]. TSLP binding to the TSLPR/IL-7Ra heterodimeric complex lead to downstream phosphorylation and activation of STAT5 in mice and STAT3 in humans [174, 175]. TSLPR expression was initially thought to be limited to immature B cells and myeloid cells such as monocytes and DCs [169, 176]. However, recent discoveries revealed a much wider pattern of TSLPR expression, including mast cells and CD4⁺ T cells [177, 178]. Myeloid DCs and mast cells have been found to express TSLPR constitutively [163, 178, 179], whereas CD4⁺ T-cell expression of TSLPR required TCR stimulation [177, 180]. Ligation of TLR2, TLR8, and TLR9 triggers TSLP production in human primary epithelial cell lines [173], and double-stranded intermediate RNA from rhinovirus also augments TSLP production by Th2 cytokines-primed epithelial cells through TLR3 [171]. Diesel particles as well as cigarette smoke also trigger TSLP production from AECs [51]. As airway viruses as well as diesel exhaust and cigarette smoke are abundant in many places in the Western world today, their ability to induce TSLP might act as a predisposing factor for allergic sensitization and inflammation [181, 182].

In humans, myeloid DCs express the highest level of TSLPR in both transcript and protein levels among hematopoietic cell lineages, suggesting that myeloid DCs are the primary responders. After the administration of TSLP to mDCs, pDCs, and monocyte-derived DCs, we found that pDCs and monocyte-derived DCs failed to show any phenotypical changes, and detectable TSLPR could not be demonstrated (Fig. 2) [183]. In contrast, human mDCs responded rapidly to TSLP by upregulating their surface expression of MHC class II, CD40, CD80, CD86, and the DC-associated activation marker, DC-lamp [163, 183] (Fig. 2). The phenotype of TSLP-maturated mDCs differs from maturation induced by other stimuli, such as CD40L and TLR ligands. TSLP-stimulated mDCs fail to produce the Th1-polarizing cytokine IL-12 and the pro-inflammatory cytokines TNF- α , IL-1 β , and IL-6. TSLP instead triggers mDC production of chemokines such as eotaxin-2, IL-8, and TARC, which are important for recruiting eosinophils, neutrophils, and Th2 cells, respectively [184]. TSLP treatment of mDCs results in the inhibition of IL-12 production even following TLR ligation [39, 185]. This suggests that TSLP is important in enabling mDCs to induce Th2 immune responses or at least to prevent them from producing Th1-polarizing cytokines.

Many of the Th2-inducing properties of TSLP-treated DCs can be associated with their upregulation of OX40L after TSLP exposure [186]. OX40L binds the OX40 receptor preferentially expressed on the surface of activated, but not memory, CD4⁺ and CD8⁺ T cells, activated CD4⁺ Treg cells, natural killer T cells, natural killer cells, neutrophils, and more recently, on human

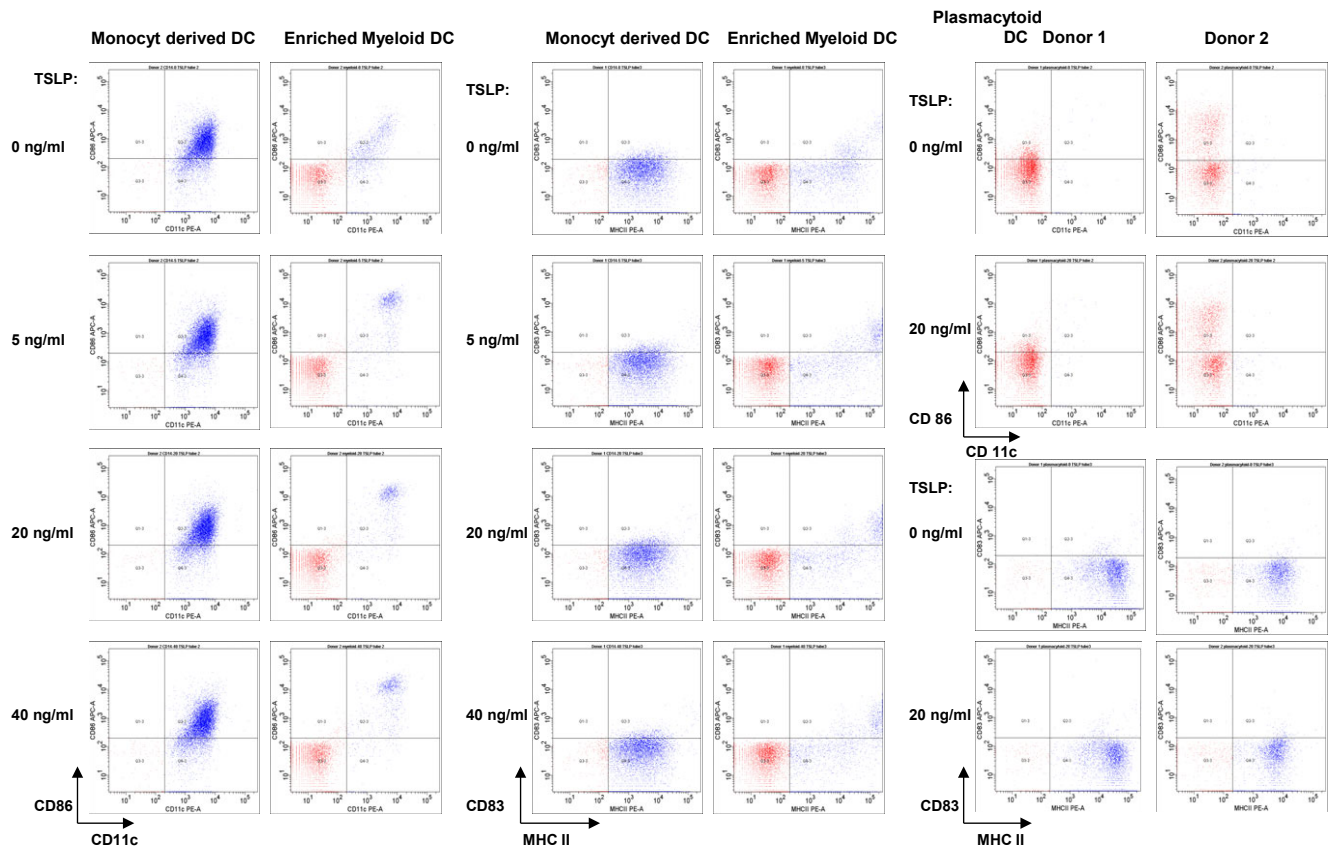


Fig. 2. Effect of TSLP administration on mDCs, pDCs, and monocyte-derived DCs. In a set-up to investigate TSLPs effect on different DC populations, monocyte-derived DCs, pDCs, and mDCs were exposed to increasing concentrations of TSLP (1–40 ng/mL) for a 24-hour culture period in RPMI with autologous serum. Expressions of CD11c, CD83, CD86, and MHC-II were determined by flow cytometry. CD11c⁺ mDC showed strong increases in mean fluorescence of CD86 as well as a slightly increased fluorescence of CD83 after TSLP administration. Expression of MHC-II was also slightly upregulated by TSLP. In contrast, monocyte-derived DCs and pDCs were largely unaffected by TSLP administration.

airway smooth muscle cells [187–189]. Unlike other co-stimulatory molecules, OX40 is not constitutively expressed on naïve T cells but induced 24 to 48 h after the engagement of TCR and peaks *in vivo* between day 2 and day 5 after antigen immunization [190]. OX40L-induced inflammatory Th2-cell differentiation depends on the absence of IL-12, as OX40L is incapable of triggering inflammatory Th2-cell differentiation in the presence of IL-12. This suggests that TSLP-activated DCs may create a Th2-permissive microenvironment by upregulating OX40L without inducing the production of Th1-polarizing cytokines.

In addition to its effects on mDCs, TSLP has also been suggested to activate human mast cells to produce Th2-cell-associated effector cytokines IL-5 and IL-13, as well as IL-6 and GM-CSF, in the absence of T cells or IgE cross-linking [170]. However, mast cells are unable to respond to TSLP alone and require either IL-1 or TNF- α to initiate cytokine expression [170], suggesting that a pro-inflammatory environment must be present for mast cells to be responsive to TSLP. Similarly,

exposure to IL-33, an IL-1 family member discussed below, also sensitizes mast cells to TSLP stimulation [179]. Interestingly, IL-13 and IgE-receptor cross-linking have been shown to induce mast cell [191, 192] and basophil [193] expression of TSLP, pointing to an important feedback loop acting via DCs to enhance Th2-cell immunity. Studies in mice have furthermore shown that epithelial or basophil-derived TSLP, in the presence of anti-CD3 stimulation *in vitro*, directly induces Th2 differentiation of naïve CD4⁺ T [178]. This has not been shown in humans, and other studies in both human and mice suggest that DCs are the primary cells responsible for TSLP-induced allergy. In addition, TSLP-activated mDCs induce a robust expansion of human Th2 memory cells while maintaining their central memory phenotype and Th2 commitments [194].

Most of the current, above-mentioned, data point to a primary role for TSLP in the sensitization/priming stage of allergic airway disease. Elevated TSLP expression has been seen in a variety of allergic inflammatory conditions, such as allergic rhinitis [195, 196], and in

response to a wide variety of allergens [197–199] and is potentially a crucial player in allergic asthma responses.

Finally, expression of TSLP is increased in bronchial biopsies and sputum from asthmatic individuals, particularly in those with severe asthma [186, 200], and increased expression of TSLP has also been correlated with asthma disease severity [200]. This is most likely a result of the reduced barrier function due to the ongoing airway inflammation seen in the epithelium of asthmatics. Taken together, these data suggest that TSLP primarily produced by the AECs represents a crucial factor, which initiates allergic responses at the epithelial cell surface.

IL-25 signalling

IL-25 (alias IL-17E) was initially described as a cytokine produced by Th2 cells but has subsequently been detected in epithelial cells, macrophages, microglia, activated eosinophils, mast cells, basophils, and CD8+ and CD4+ cells [156, 201, 202]. Unlike TSLP and IL-33, the two other main epithelial-derived cytokines promoting Th2 responses, IL-25 is expressed by both T cells and APCs. Exposure to allergens and air pollutants increases IL-25 secretion maybe primarily from eosinophils and basophils but also from AECs, and cells obtained from asthmatics produce increased levels of IL-25 after allergen activation [203, 204].

Studies in mice showed that systemic administration or overexpression of IL-25 led to increased levels of Th2 cytokines and eotaxin, resulting in eosinophilia, increased serum IgE, and mucus hyperplasia [80, 205, 206]. Administration of neutralizing antibody against IL-25 in allergic asthma models significantly reduced levels of IL-5, IL-13, and serum IgE, combined with decreased levels of Th2 cells and eosinophils [207]. IL-25 was shown to aid stimulation and proliferation of Th2 memory cells and, by an IL-4 independent mechanism, to further enhance Th2 cytokine production, in particular IL-5 [204]. Taken together, human *in vitro* studies and *in vivo* studies in mice show that IL-25 amplifies allergic immune responses and that IL-25 plays an important role in the development of Th2-mediated allergic inflammation. Moreover, because IL-25 is also produced by activated eosinophils, basophils, and mast cells, it may play a critical role in the maintenance and re-stimulation of allergen-specific Th2 memory cells expressing IL-25R.

IL-33 signalling

IL-33 is a member of the IL-1 family and was identified as a ligand for ST2 (alias IL-1RL1), a receptor stably expressed in Th2 cells [208, 209]. IL-33 is expressed by

nonhematopoietic tissue cells such as fibroblasts and epithelial and endothelial cells [210]. The ST2 receptor is closely related with IL-1R1 and IL-18Ra [211], but binds to neither IL-1 nor IL-18. ST2 is expressed on activated Th2 cells and mast cells independently of IL-4 activation [212], which suggests that IL-33 plays a role in augmenting Th2 cytokine responses *in vivo*. Incubation of basophils with IL-33 upregulates their production of IL-9, whereas Th2-polarized cells cultured with IL-33 increase their production of IL-5, IL-9, and IL-13, while IFN- γ levels are decreased [162, 213].

ST2, as well as the IL-25 receptor IL-17RB, has also been identified on human and murine ILC2s. The ILC2s are activated to produce type 2 cytokines such as IL-5, IL-9, and IL-13 in response to IL-33 and IL-25 [83, 84, 90]. Over half of all IL-5- and IL-13-producing cells in the lungs after intranasal administration of IL-25 or IL-33 are ILC2 cells, as discussed previously. Other studies have found that only IL-33 can activate lung ILC2s, whereas IL-25 and TSLP only enhance cytokine production [214]. There also appear to be some differences in how ILC2s are activated depending on the tissue in which they reside as ILC2 cells isolated from human skin have been shown to mainly be activated by TSLP and are largely impervious to the activation by IL-25 and IL-33 [91].

Treating human mast cells or CD34+ mast cell progenitors with IL-33, but not IL-18, increases their survival and cytokine production [179, 215]. Although TSLP augments the effects of IL-33 on mast cells [179], TSLP alone is not capable of inducing cytokine production by mast cells, indicating that IL-33 increases responsiveness to TSLP, potentially through upregulation of TSLPR [179, 216]. AECs have been found to release IL-33 in response to allergen exposure [217], and IL-33 expression is increased in the airways of patients with severe asthma [218]. The induction of a Th2 cytokine response by IL-33 is linked to increased goblet cell hyperplasia, mucus secretion, and eosinophilia [162]. IL-33 signalling has also been shown to be necessary in the induction of experimental HDM-induced allergic asthma and anaphylaxis to peanut in mice [219]. This effect was most likely due to the induced expression of OX40L on DCs, and although TSLP and IL-25 can induce OX40L as well, only IL-33 signalling was necessary for an intact allergic response [219]. Other animal studies show that blocking IL-33, during sensitization to inhaled antigens, abolishes Th2 development [220], further suggesting that IL-33 is an essential cytokine in Th2 responses. However, this is in contrast to other studies showing that IL-33 does not appear to be required for the initiation of Th2 cytokine-mediated responses *in vitro* [221] or *in vivo* [222] and so, further studies are needed to determine the impact of IL-33 on Th2 differentiation.

Cross-regulation and synergy of IL-25, IL-33, and TSLP

TSLP, IL-25, and IL-33 each separately promote Th2 cytokine responses, and their regulation and effect on target cells are intertwined, often creating positive synergistic effects that further amplify Th2-mediated inflammation. In terms of direct regulation of expression, both IL-25 and IL-33 induce TSLP production by AECs [156, 223], suggesting that IL-25 and IL-33 are both upstream of TSLP. This is further supported by the observation that TSLP enhances release of Th2 cytokines by mast cells only when co-stimulated with IL-33, IL-1, or TNF [161, 170, 179].

Furthermore, IL-33, but neither TSLP nor IL-25, elicits increased cytokine production from basophils [213, 224]. IL-25 and IL-33 both upregulate their own cognate receptor, which suggest a positive feedback loop to further amplify innate cells responsiveness to TSLP, IL-25, and IL-33 [205, 225]. Additionally, TSLP, IL-25, and IL-33 all upregulate IL-25R, which indicates that exposure to IL-25 is an important aspect of these AEC-derived cytokines [204, 205, 225]. Differentiated Th2 cells are also sensitive to IL-33 stimulation, which increases their secretion of IL-5 and IL-13 [162]. Neither IL-25 nor TSLP appears to regulate IL-33 or IL-33 receptor expression.

GM-CSF, IL-1 β , and TNF- α

GM-CSF is another cytokine produced by AECs that affects DC maturation in the lungs [226, 227]. Studies in mice show that GM-CSF is capable of breaking inhalation tolerance and that blocking of GM-CSF neutralizes HDM-driven asthma [228]. Epithelial cells cultured from patients with asthma continually overproduce GM-CSF [229], and exposure to allergen proteases induces secretion of GM-CSF by epithelial cells [107, 230]. AECs also respond to the Th2 cytokines IL-4 and IL-13 by producing GM-CSF as well as IL-8, CCL-11, and CCL-17 further propagating the inflammatory Th2 pathogenesis [231] by attracting neutrophils, eosinophils, and CD4⁺ Th2 cells, respectively.

IL-1 β and TNF- α initiate inflammation and innate immune responses, but they also play key roles in epithelial interactions with DCs in allergic asthmatic responses. Studies in mice show that TNF- α breaks tolerance to inhaled allergens through the activation of lung DCs [100]. Stimulating AECs with known asthma exacerbators, such as allergens, cigarette smoke, and diesel exhaust particles, induces increased secretions of TNF- α and GM-CSF potentially skewing DCs towards the promotion of Th2 inflammatory responses [227]. TNF- α and IL-1 β can synergize with Th2 cytokines (IL-4 and IL-13) to induce TSLP production by human epithelial cells and keratinocytes [172]. As mentioned

above, these exacerbators also induce TSLP production by AECs, and TNF- α and IL-1 β are also known as strong inducers of epithelial secretion of TSLP and GM-CSF [173], suggesting a strong positive feedback loop in the airways between all these Th2-inducing cytokines.

Modulation of DC and T-cell function by AEC-derived uric acid

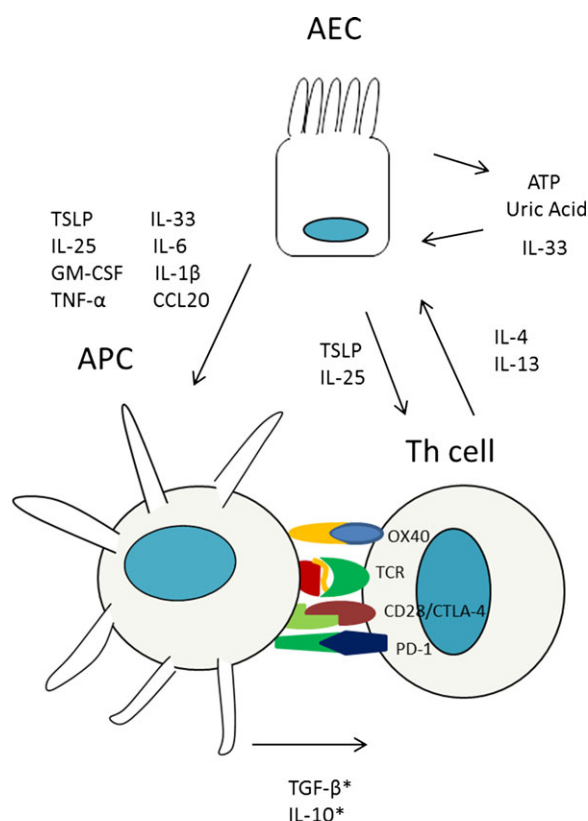
Recent studies have shown that ATP and uric acid potentially play important roles in allergic reactions. After a single administration of HDM in the airways of naive mice or to patients with atopic asthma, both ATP and uric acid production are induced, resulting in the activation of DCs and other innate immune cells, presumably via the activation of purinergic receptors on epithelial cells [232, 233]. HDM-induced uric acid promoted Th2 sensitization by amplifying the epithelial production of TSLP, GM-CSF, and IL-25. Moreover, the AECs were found to be the source of uric acid through

TLR4 signalling, and the epithelial production of uric acid was later found to be a response to reactive oxygen species (ROS), after inhalation of allergens, virus, or environmental pollutants [234].

Uric acid has also been associated with the effects of the vaccine adjuvant alum. A number of studies revealed that alum acts via activation of the NLRP3 inflammasome [235–237], a multiprotein oligomer containing NLRP3 (NACHT, LRR, and PYD domain-containing protein 3) and caspase-1 that matures cytokines such as IL-1 β and IL-33 for secretion [238]. Studies in mouse models show that allergic airway inflammation depends on NLRP3 inflammasome activation and that DC recruitment to lymph nodes is reduced in the absence of NLRP3 [239]. Absence of NLRP3 resulted in reduced levels of pro-inflammatory Th2 cytokines: IL-5, IL-13, IL-33, and TSLP [239]. Interestingly, uric acid has been reported to activate the NLRP3 inflammasome [240] even though administration of uric acid with antigen has also been reported to facilitate Th2 immunity and features of asthma in the absence of NLRP3 inflammasome [237]. These findings provide a molecular insight into Th2-cell responses and identify uric acid as an important initiator and amplifier of allergic inflammation in the airways (Fig 3).

Mechanism of AEC/DC inhibition of T-cell responses

In allergic diseases, there is evidence that suppression of Th2 responses to allergens by both inducible Tregs and Tr1 cells is deficient [241, 242]. Naturally occurring Tregs are also reduced in bronchoalveolar lavage samples from patients with asthma compared with those seen in control subjects [243, 244]. Several cytokines



* Molecules involved in inhibitory responses, triggered by AEC interactions

Fig. 3. Main cellular targets of AEC cytokines in allergic Th2 responses. An overview of AEC-derived cytokines with defined target cells discussed throughout this review. The majority of the cytokines directly affect APC function by influencing APC migration and activation, leading to inflammatory Th2 responses, and Th2-derived cytokines are able to provide positive feedback to inflammatory AEC cytokine production. Conversely, APC contact with healthy AECs is able to downregulate T-cell responses. Furthermore, activation of IL-25R and IL-33 receptor ST2 on AECs induces TSLP production, and IL-25 and IL-33 both upregulate their own cognate receptor on AECs and innate cells, suggesting a positive feedback loop to further amplify innate cells responsiveness to TSLP, IL-25, and IL-33. TSLP, IL-25, and IL-33 all upregulate IL-25R, which indicates that IL-25 has a key role in mucosal immunology. Other cell types like ILC2 and even mast cells are also affected by AEC-derived cytokines and may be involved in the shaping of mucosal Th2 responses, but these have been left out for simplicity.

and other signalling molecules such as IL-10, TGF- β 1 and the vitamin D3 metabolite 1,25-dihydroxyvitamin D3 have been shown to induce tolerogenic DCs and inhibit inflammatory T-cell responses [245]. Another mechanism by which DCs inhibit inflammatory T-cell responses is by the secretion of IDO, which depletes tryptophan and halts T-cell growth [246].

In addition, both birch pollen and HDM challenges induce certain immune responses in healthy individuals that are not seen in allergic patients [121, 247–249],

suggesting that allergy might at least partially be caused by a lack of a protective tolerogenic immune response [250].

Induction of tolerogenic T-cell responses in the airways

The place of induction of the Foxp3⁺ Tregs and Tr1 cells required for immune tolerance in the respiratory tract is still not fully defined [251], but the process depends on airway DCs migrating to the draining bronchial lymph nodes [252]. As discussed above, the ability of DCs to polarize T-cell responses is determined by signals received when DCs are immature, and in relation to allergy and asthma, these signals are often influenced by cytokines derived from the airway epithelium. Investigations of DC maturation and polarization have largely focused on the effect of the AEC-derived cytokines discussed above, but cell-to-cell interactions with the epithelium may also instruct DC responses. Our group has shown that after LPS exposure, DCs in direct contact with intact and polarized AECs exhibit a more tolerogenic phenotype, and we have hypothesized that DCs in direct contact with an intact epithelium are instrumental in the control of hyper-immunogenic responses towards allergens [38].

Similar studies with DCs together with AECs, but also intestinal ECs, have also shown that these EC-imprinted DCs have a decreased ability to activate T cells, mainly through the production of IL-10 [128, 130, 253]. Corresponding studies with murine AECs have also revealed increased DC secretion of IL-10 together with an expression of inhibitory receptor PD-L2, lowered expression of CD40 and CD86, and lowered production of TNF- α and IL-12p40 after exposure to AECs [130, 254]. These findings correspond well with our results using AECs, wherein the observed tolerogenic DCs also increased expression of co-inhibitory receptor ILT3 and decreased expression of eotaxin, MCP-1, and IL-12 [38]. We have further showed that DCs that have been in direct contact with fully polarized AECs significantly decrease T-cell proliferation when co-cultured with autologous birch allergen-specific T-cell lines [38] and grass and HDM allergens [37]. The decreased T-cell proliferation was coupled with decreased levels of the Th2-specific cytokines IL-5 and IL-13, showing that the AEC-imprinted, birch allergen-loaded DCs had lost some of their ability to stimulate memory T-cell responses. Similarly, co-culturing AEC-imprinted DCs with freshly isolated CD4⁺ T cells from birch-, HDM-, or grass-allergic individuals decreased their ability to proliferate when re-stimulated with relevant allergens [37]. However, no induction of Tregs or any increased production of IL-10 from either T cells or DCs was observed, indicating that this was an IL-10-independent mechanism. In contrast, epithelial imprinting in the

intestines seems to involve tolerance based on IL-10-producing Treg [39].

In our investigations, these regulatory effects were only observed with DCs that had been in direct contact with AEC. Other studies with both primary AECs and cell lines have shown that AEC-imprinted APCs can induce regulatory T-cell populations and inhibit inflammatory Th2 proliferation – after both direct contact with AECs and only in response to AEC supernatant [119, 130, 255].

Closing remarks

The aim of this review has been to bring up-to-date information on the influence of the airway epithelium on ensuing T-cell responses in relation to asthma and allergy. It discusses how allergens or other environmental agents directly induce changes in the airway epithelium, affecting neighbouring cells such as the DCs, ILC2s, mast cells, and basophils, to how these changes directly affect T-cell responses. Both genetic and environmental factors are most likely involved in the pathogenesis of these airway diseases, as well as the ability of particular allergens to stimulate the innate immunity, which is required to break tolerance. In the last decade, it has become increasingly clear that the airway epithelium is an important player in allergic inflammation, remodelling, and bronchial hyper reactivity. The activation of AECs with different antigens/allergens not only induces the expression of chemokines resulting in the recruitment of immature DCs, but also creates a micro-environment, which instructs resident DCs to polarize adaptive T-cell responses. TSLP, IL-25, IL-33, and many other AEC-derived cytokines play a critical role in the

initiation of Th2-cytokine responses at mucosal sites (see Fig. 3).

The coordinated expression of these chemokines and cytokines, coupled with the cross-regulation and expression of their ligands and receptors, suggests that a complex interplay exists between AECs and neighbouring innate cells such as DCs and ILC2s. Whereas a dysfunctional epithelium plays an important role in the pathogenesis of allergic inflammatory Th2 responses, an intact and healthy epithelium appears to be just as crucial in upholding homeostasis. Studies with both airway and intestinal epithelial cells have illustrated their regulatory and tolerance-inducing capacity. Whether this tolerance is active through activation of Tregs or passive tolerance through 'failed' activation of inflammatory T-cell responses is still unclear. Epithelial cells have been found to induce production of IL-10 and TGF- β from monocyte-derived DCs, which are both crucial cytokines in the induction of Tregs and Tr1 cells. However, our studies on birch-, grass-, and HDM-allergic patients show that DCs that have been in contact with AECs directly reduce the induction of inflammatory T-cell responses towards allergens. A better understanding of the interactions between AECs and neighbouring innate cells and how these interactions can be skewed to promote either inflammatory or inhibitory immune responses may provide crucial knowledge for not only the development of future therapeutic treatments, but also the prevention of allergic airway disorders.

Conflict of interest

The authors declare no conflict of interest.

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