

Asthma: The importance of dysregulated barrier immunity

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Chronic asthma is an inflammatory disease of the airway wall that leads to bronchial smooth muscle hyperreactivity and airway obstruction, caused by inflammation, goblet cell metaplasia, and airway wall remodeling. In response to allergen presentation by airway DCs, T-helper lymphocytes of the adaptive immune system control many aspects of the disease through secretion of IL-4, IL-5, IL-13, IL-17, and IL-22, and these are counterbalanced by cytokines produced by Treg cells. Many cells of the innate immune system such as mast cells, basophils, neutrophils, eosinophils, and innate lymphoid cells also play an important role in disease pathogenesis. Barrier epithelial cells are being ever more implicated in disease pathogenesis than previously thought, as these cells have in recent years been shown to sense exposure to allergens via pattern recognition receptors and to activate conventional and inflammatory-type DCs and other innate immune cells through the secretion of thymic stromal lymphopoietin, granulocyte-macrophage colony stimulating factor, IL-1, IL-33, and IL-25. Understanding this cytokine crosstalk between barrier epithelial cells, DCs, and immune cells provides important insights into the mechanisms of allergic sensitization and asthma progression as discussed in this review.

Keywords: Asthma · Lung inflammation · T cells

Adaptive immunity in asthma: more than a Th2-mediated disorder

Chronic asthma is an inflammatory disease of the airway wall. The earliest studies on asthma pathology found that CD4⁺ T lymphocytes were present in asthma biopsies. Over the past 30 years, the Th1–Th2 paradigm has dominated the asthma research field. The immune response to inhaled allergens (such as house dust mite (HDM), cockroach, pollen grains, or fungal spores) is characterized by an aberrant Th2 lymphocyte response that has the potential to cause the features of asthma. Th2-type cytokines cause airway eosinophilia (IL-5), goblet cell metaplasia (GCM; IL-4 and IL-13),

and Bronchial hyperreactivity (BHR) (IL-4 and IL-13), all salient features of asthma (reviewed in [1]). BHR is the tendency of the airways to overreact to all kinds of nonspecific stimuli such as cold air and exercise. Animal models of asthma, in which these Th2-type cytokines have been individually neutralized, illustrate the importance of cytokines in promoting allergic-type airway inflammation. IL-4-deficient mice are deficient in IgE synthesis and have been shown to be protected from developing asthma through defects in eosinophil recruitment [2]. Most of the effects of IL-4 can be mimicked by IL-13 and, not surprisingly, IL-13-deficient mice develop neither BHR nor GCM [3, 4]. IL-5-deficient mice do not develop airway or bone marrow eosinophilia, and eosinophil-deficient mice show defects in airway wall remodeling, which is another feature of persistent asthma [5]. Adoptive transfer studies of in vitro generated OVA-specific Th2 cells also demonstrate that Th2 cells are sufficient to induce

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most features of asthma, such as BHR, airway eosinophilia, and GCM [6].

Although it was initially thought that Th2 cytokines are mainly produced by adaptive immune cells, studies using reporter mice have revealed that many cells participating in the ongoing airway inflammation, such as invariant NKT cells, basophils, eosinophils, mast cells, type 2 innate lymphoid cells (ILC2s), and myeloid cells can also produce the Th2-cell-associated cytokines IL-4, IL-5, and IL-13 [7–9]. Furthermore, the view that asthma is an exclusively Th2-dominated disease has been challenged by the discovery that other cytokines such as IL-9, IL-17, and IL-22 are frequently found co-expressed with Th2 cytokines in the airways of mouse models of asthma or in humans with asthma. In addition, in humans with asthmatic airway inflammation, a Th2-biased response can only be seen in 50% of patients [10, 11] and clinical trials with inhibitors of Th2 cytokines have shown benefits in only a small subset of patients [12, 13]. Some human asthmatics have predominantly neutrophilic airway inflammation and the presence of airway neutrophils is correlated with a worse outcome, and with a lower response to inhaled steroids, the standard therapy for moderate-to-severe asthma. Neutrophils are probably recruited to the airways by IL-17-producing cells that simultaneously produce IL-4 [14]. Therefore, the classical view of asthma as a Th2-driven disease can be modulated when the roles of the following cell types is considered.

The role of innate immune cells in asthma

The fact that eosinophil-rich responses could be induced in mice lacking T and B cells suggested a potential role for the innate immune system during allergic immune responses (reviewed in [15]). Initially the cell type involved was vaguely called a non-T non-B cell, but these cells have been renamed as ILC2s [16]. Murine ILC2s express CD127, Sca-1, T1/ST2 (the receptor for IL-33), and IL17RB, the receptor for IL-25. When activated by cytokines, such as IL-25 or IL-33, ILC2s can control some of the features of asthma including BHR, goblet cell hyperplasia, and eosinophilia through the production of IL-5, IL-9, and IL-13 [9, 17–23] (Fig. 1). In mice, ILC2s derive from committed T1/ST2⁺ pre-ILC2s that develop from common lymphoid progenitors in the bone marrow under the influence of IL-33 and/or IL-25 but not thymic stromal lymphopoietin (TSLP). Strikingly, T1/ST2⁺ ILC2, and pre-ILC2s can be identified in Gata3-reporter mice [24, 25]. Recent breakthrough studies have identified the master transcription factors for ILC2 development in mice as being ROR- α and GATA3, which should allow more detailed study of the development of these cells [26–28]. Several allergens (house dust mite, *Alternaria*, papain), as well as nematodes that transit through the lungs, have been shown to induce ILC2 recruitment and/or proliferation in the lungs [17, 20]. Viral exacerbations of asthma (modeled by influenza virus infection in mouse models of asthma), by inducing IL-33 production by macrophages, can also lead to BHR via IL-13 production by ILC2s [19]. The pre-

cise signals involved in the recruitment of ILC2s to inflammatory sites are currently unknown, but mRNA expression data suggest that the same chemokine receptors that attract Th2 cells to the lungs (CCR4, CCR8, and CCR2) might be involved. As production of the CCR4 ligands, TARC and MDC, depends on STAT6 signaling in epithelial cells, the latter finding explains why ILC2 accumulation depends on STAT6 [29]. The signals that dampen ILC2 recruitment are only now being recognized although lipoxin A4 is a resolvin that has been shown to suppress ILC2 accumulation in the lungs of human asthmatics [30]. One caveat to all the above-mentioned studies, however, is that most experiments were conducted in mice on an RAG background and thus in mice that essentially lack an adaptive immune system, thereby potentially overestimating the importance of ILC2s in eosinophil recruitment.

Epithelial cytokines control adaptive and innate immunity to allergens

Initially thought of as merely representing the first line barrier to inhaled antigens, it is now clear that bronchial epithelial cells are a decisive layer that controls many aspects of pulmonary immunity (reviewed in [31]). Epithelial cells influence adaptive immunity by affecting the function of antigen presenting cells (APCs). Before the adaptive immune system can respond to inhaled allergens, the allergens have to be presented to them by professional APCs such as macrophages, B cells, dendritic cells (DCs) or even by less professional APCs such as basophils and eosinophils [32, 33]. We have recently created TCR transgenic mice reactive to an immunogenic peptide of Der p 1, one of the major allergens of the HDM *Dermatophagoides pteronyssinus*, to address which APCs present inhaled allergens to naive CD4⁺ T cells in the draining mediastinal LNs of the lung [34]. Using this novel tool, only mucosal lining DCs were able to present HDM-derived antigens to T cells in the mediastinal nodes, whereas B cells or macrophages were unable to do so. These results are consistent with other reports demonstrating that only DCs, but not basophils, are able to induce Th2 immunity to HDM upon adoptive transfer to naive mice, and that CD11c^{hi} cells (depleted via the CD11cDTR system) are necessary for the development of Th2 immunity to HDM allergens [8].

It is well established that DCs play a role both in the initiation and maintenance of allergic airway inflammation and asthma, and control many aspects of the disease, including BHR and GCM. DCs do so by controlling the recruitment and activation of Th2 cells, by producing chemokines that attract eosinophils and Th2 cells, and by expressing co-stimulatory molecules for terminal Teff-cell generation (reviewed in [35]). The exact subtype of DCs exerting all these functions is a matter of intense study [36, 37]. In our hands, Th2 priming was mainly performed by CD11b⁺ conventional (c)DCs, and not by CD103⁺ cDCs [34]. The restimulation of Th2 effector cells and recruitment of inflammatory cells was the function of CD11b⁺ CD64⁺ Fc ϵ RI⁺ monocyte-derived DCs [34]. In our previous work, we have found that plasmacytoid DCs

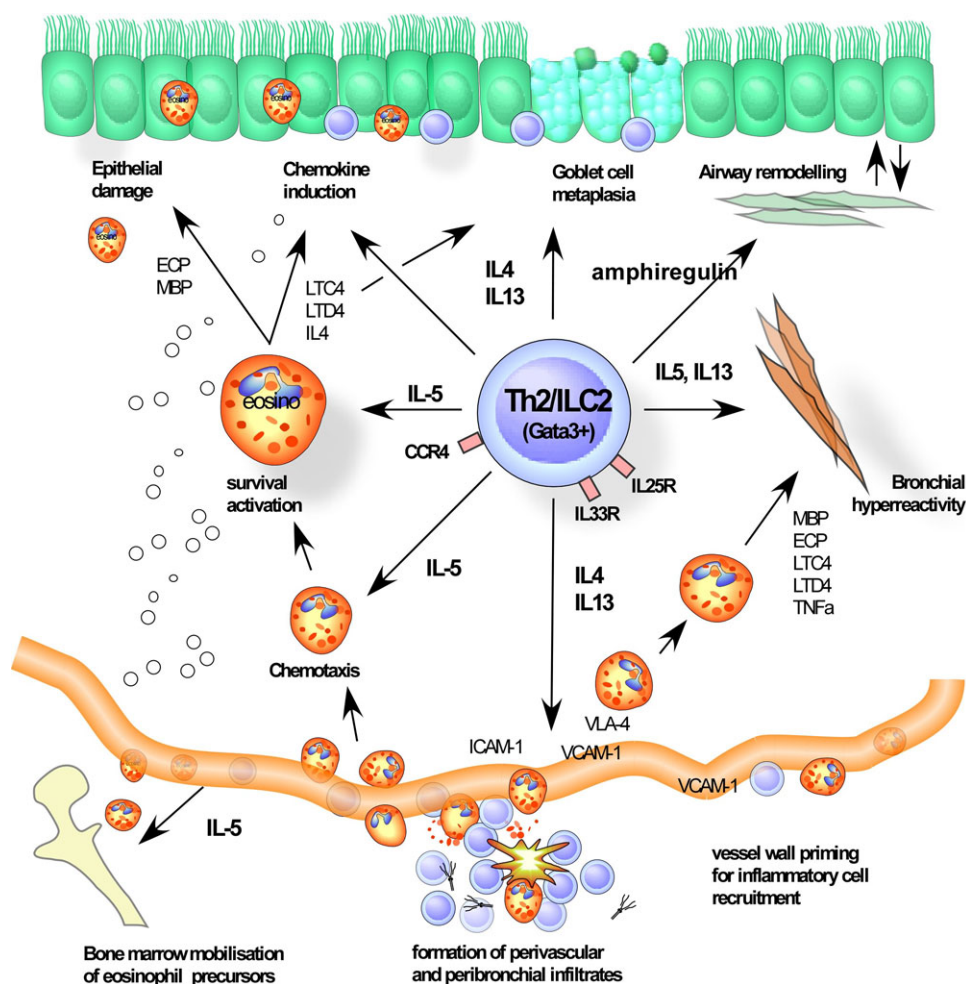


Figure 1. Control of allergic-type inflammation by Th2 cells and innate lymphoid cells type II (ILC2s). Many of the features of asthma can be controlled by cytokines made by Th2 cells and ILC2s. Interleukin-5 controls the development and activation of eosinophils. IL-4 controls GCM and causes the upregulation of cell adhesion molecules on the vessel wall. IL-13 can also cause GCM and triggers BHR, the tendency of the bronchial smooth muscle to contract to nonspecific stimuli. After eosinophils are recruited, they contribute to damage of the epithelium and cause BHR by releasing eosinophil cationic protein, major basic protein, and leukotrienes (LTC4).

induced anti-inflammatory effects and prevented asthma development, possibly by activating Treg cells [38,39].

As epithelial cells represent the first line of defense to inhaled allergens and also express TLRs, they have the ability to sense the same stimuli as innate immune cells. Triggering of these epithelial cell pattern recognition receptors (PRRs) by PAMPs initiates NF- κ B activation and leads to the release of pro-Th2 cytokines such as TSLP, granulocyte-macrophage colony stimulating factor (GM-CSF), IL-1 α , IL-25, and IL-33 in mice [40–42]. These cytokines all share the capacity to activate DCs, which then coordinate the subsequent Th2-type immune response. DCs can, however, also be directly activated by stimulation of their PRRs. Additionally, PRR-dependent epithelial cell activation also results in the production of endogenous danger signals such as uric acid, adenosine triphosphate, and lysophosphatidic acid [43]. Furthermore, IL-4 and IL-13, produced by Th2 cells or ILC2s, have the potential to induce TSLP, GM-CSF, and CCL20 production by human air-

way epithelial cells [44]. It is therefore likely that IL-4R- α expression on airway epithelium might represent an important feedback mechanism through which IL-4 and IL-13-secreting immune cells enhance Th2-cell immunity in ongoing immune responses.

Cytokines secreted at the barrier that control Th2 immunity to allergens

Interleukin 1 α and IL-1 β are among the first described members of the prototypical IL-1 cytokine family that also includes IL-18, IL-33 (IL-1F11), and many others. IL-1 β is synthesized as a pro-form that requires cleavage via the inflammasome-caspase-1 axis to be secreted as a biologically active cytokine. There is renewed interest in the role of IL-1 and related cytokine family members in promoting asthmatic airway inflammation, due to new evidence in HDM-driven models of asthma, as well as to genetic polymorphism

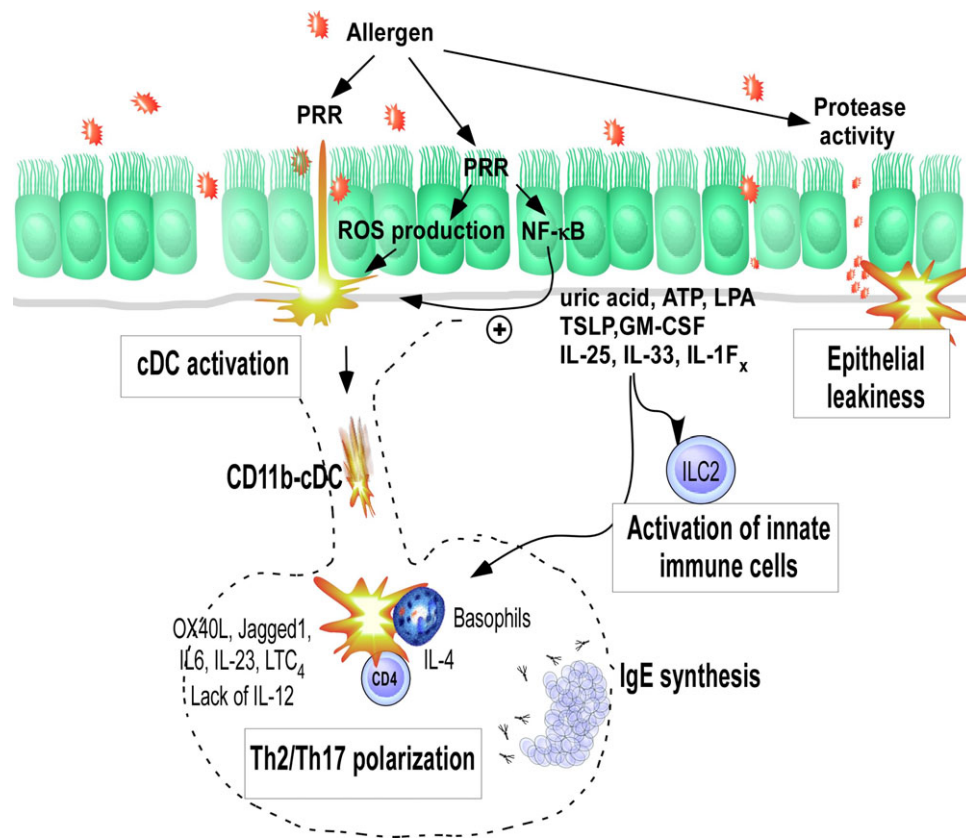


Figure 2. Mechanism of allergic sensitization. When allergens are inhaled, they can trigger various PRRs on epithelial cells and dendritic cells (DCs). This leads to the production of ROS and activation of NF- κ B signaling in epithelial cells, and subsequently to production of various mediators such as uric acid, adenosine triphosphate, lysophosphatidyl acid, TSLP, GM-CSF, and various interleukins, including interleukin-1 family members. These endogenous danger signals and cytokines lead to activation of innate lymphoid cells (ILC) and conventional DCs (cDC) that move to the mediastinal nodes to induce Th1 and Th17 differentiation. Basophils also get activated and collaborate in the LN with cDCs to induce Th2 immunity by releasing IL-4.

studies in human cells [45]. Indeed, initially it was thought that IL-1 played only a minor role in asthma, as symptoms in the classical OVA-alum model of asthma were not reduced in IL-1R-deficient mice. [46, 47]. Using radiation-induced bone marrow chimeric mice and exploiting the natural route of pulmonary exposure to HDM allergen, we have recently found that IL-1R triggering on radioresistant lung epithelial cells promotes the innate immune response to natural allergen [41]. Autocrine release of IL-1 α by HDM-exposed bronchial epithelial cells leads to TSLP, GM-CSF, and IL-33 production by epithelial cells, and IL-1 α is required for the development of Th2 immunity to HDM in vivo (Fig. 2) [41]. It is still unclear whether the inflammasome-caspase1-IL-1 α axis is involved in asthma development as one group failed to see an effect of Nlrp3 deficiency on asthma development in their mouse model whereas other groups found a role when allergens were introduced via the skin or alum was used as an adjuvant [43, 48, 49].

Interleukin-33 has been shown to act upstream of the type-2 effector cytokine cascade, by stimulation of various innate and adaptive immune cells, and by inducing the apoptosis of lung epithelial cells. Allergic asthma patients express higher levels of IL-33, as determined by mucosal biopsies, as compared with those

of healthy subjects, and genetic association studies have identified SNPs in the IL-33 and IL-33R (T1/ST2) locus associated with asthma [50, 51]. In mice, neutralization of IL-33 blocks development of lung Th2 immunity to a number of allergens, such as HDM and peanuts, as well as to lung-dwelling parasites such as hookworms [41, 52, 53]. Numerous cells of the innate immune system, such as DCs, macrophages, basophils, mast cells, and eosinophils express T1/ST2 (the receptor for IL-33) and stimulation of these cells by IL-33 leads to prolonged survival and/or activation, often leading to increased Th2 immunity in mouse models of allergy and asthma [50, 52, 54–57]. Little is known, however, about the mechanism of IL-33 release from epithelial cells, endothelial cells, fibroblasts, and immune cells [58]. IL-33 is possibly released in a passive manner as a result of necrotic cell death, acting as an alarmin. During necrosis, IL-33 remains in its active form whereas, under conditions of apoptotic cell death, the executor caspases, caspase-3 and caspase-7, cleave IL-33 into an inactive form [59]; however, in fibroblasts, IL-33 can also be released in an active process triggered by mechanical stretching. No studies have so far reliably identified apoptosis or necrosis in the lungs of asthmatics, although cell death can regulate the release of IL-33 in asthma [60].

In neutrophils, pro-IL-33 can also be processed into a functionally more mature form via the action of neutrophil elastase and cathepsin G, and subsequently released [61]. Clearance of apoptotic cells, following allergen exposure, in bronchial epithelial cells requires Rac1, which leads to a suppression of IL-33 production in a process requiring IL-10 in mice [62]. In an HDM-driven murine model of asthma, the epithelial repair factor Trefoil factor 2 has been shown to induce IL-33 production in airway epithelia, alveolar macrophages, and Fc γ RI⁺ inflammatory DCs and thus to contribute to the induction of Th2 immunity, in a process requiring the chemokine receptor and putative TTF2 receptor CXCR4 [53]. In virally induced airway inflammation, a typical cause of asthma exacerbation, alveolar macrophages produce large amounts of IL-33 [19]. It also appears that TLR4 and IL-1R signaling on epithelial cells occurs upstream of epithelial IL-33 release in asthma [40, 41]. The expression of T1/ST2 is itself subject to tight control through ubiquitination. As for many other cytokine receptors, ligand binding induces downregulation of surface T1/ST2. The F-box protein FBXL-19 is an orphan member of the Skp1-cullin-F box family of E3 ubiquitin ligases that binds to T1/ST2 and mediates its degradation by the proteasome, partially through the activity of GSK3 kinase [63]. It is currently unknown whether T1/ST2 is differentially ubiquitinated in asthmatics, or if the levels of FBXL-19 are modified in asthmatics versus healthy control subjects, and could be influenced by drugs and therefore be a therapeutic option for asthma.

Interleukin-25 is released by bronchial epithelial cells and airway inflammatory cells of allergen-challenged mice and humans (Fig. 2, [64–66]). The proteolytic enzyme MMP7 released from bronchial epithelial cells is necessary for the optimal production of IL-25 [67]. Although IL-25 promotes Th2 immunity in the lung in mice [68, 69], its potential to activate DCs remains unclear. Epithelial-derived IL-25 induces Jagged 1 expression on DCs and leads to Th2 responses in the lung of RSV-infected mice [70]. Furthermore, IL-25 induces IL-9 production by Th9 cells, via the IL-17RB subunit [71]. When administered via the airways, IL-25 acts directly on pre-ILC2s to induce their expansion and activation [9]. Recently, it was shown that IL-25 also expands a population of granulocytic myeloid cells that produce IL-5 and IL-13 and contribute to asthmatic lung pathology in mice and humans [72]. Epithelial IL-25 also acts directly on fibroblasts and endothelial cells to promote airway remodeling and angiogenesis and boosts production of TSLP and IL-33, thereby amplifying Th2 immunity in the lung [73].

GM-CSF, when overexpressed in the lungs of mice via adenovirus, induces spontaneous Th2 sensitization to the inhaled innocuous protein OVA, via activation of DCs [74, 75]. Moreover, epithelial cells of human asthmatics continually overproduce GM-CSF when cultured for many passages, suggesting (epi)genetic regulation of GM-CSF expression in asthmatics [76]. Conversely, neutralization of GM-CSF in mice abolishes sensitization to HDM and attenuates the adjuvant effects of diesel particles on allergic sensitization [41, 77–79].

TSLP overexpression in murine bronchial epithelial cells boosts Th2 immunity in the lungs [80]. However, in mouse models of

asthma, driven by natural allergens, the neutralization of TSLP does not necessarily lead to reduced disease [41, 52]. The expression of TSLP has been found to be increased in human asthmatics, particularly in severe disease, as measured in bronchial biopsies and sputum, as compared with levels in healthy controls [81, 82]. Genetic polymorphisms in the promoter region of human TSLP are associated with increased risk of asthma [83]. In vitro, proteolytic allergens, diesel exhaust particles, and cigarette smoke induce epithelial production of TSLP that causes DC activation [84, 85], as does LPS priming [86]. TSLP promotes the growth and differentiation of basophils from the bone marrow [87]. TSLPR is not only expressed by human DCs but also by human bronchial epithelial cells, and TSLP stimulates the proliferation of bronchial epithelial cells and IL-13 production by these cells [82]. Whether this is true in mice remains to be studied.

Role of Th17 cells in asthma

Asthma was initially proposed to be a disorder exclusively driven by Th2 cytokines. The recent emergence and characterization of the Th17 lineage of cells has, however, greatly refined the existing model of asthma, and most groups now describe the occurrence of different subsets Th cells in this disease. Co-transfer of antigen-specific Th17 cells with Th2 cells boosts eosinophilic airway inflammation in mice, and this effect is also observed by overexpression of IL-23, acting to increase the number of Th17 cells [88]. This pathway of Th17 immunity appears to be triggered when allergens are introduced via the airways directly, in contrast to the often-used OVA model, where antigen sensitization occurs via the peritoneal cavity, and could be driven by a complement 5a (C5a)-driven induction of IL-23 and/or TGF- β production by airway DCs [89–91]. As Th17 cells make many different cytokines (CD4⁺ T cells producing IL-17A, IL-17F, IL-17A/F, and/or IL-22), the precise role of individual Th17 cytokines involved in asthma is a matter of intense study. A further complicating factor is that some of the features of asthma are regulated by Th cells that produce both IL-4 and IL-17 [14].

In certain mouse models of airway inflammation, such as those driven by HDM allergen or ozone, IL-17A controls BHR and airway remodeling but did not affect airway eosinophilia and Th2-cell recruitment to the airways, and some of the pathogenic effects of IL-17 are mediated directly on bronchial smooth muscle cells and local fibroblast progenitors [91–95]. Moreover, IL-17A can induce steroid insensitivity in bronchial epithelial cells [96]. In some situations, IL-17 counteracts the immunoregulatory and anti-inflammatory effects of Treg cells, thus increasing inflammation and BHR [95]. Upon exposure to fungal spores, IL-23 and IL-17A can also dampen inflammation, in a pathway requiring TLR6 and IL-23 expression in lung DCs in mice [97, 98]. This pathway might be clinically relevant given the association between TLR6 SNPs and the risk of asthma in humans [99].

The cytokine IL-22 is increasingly implicated in controlling immunity at barrier surfaces, by inducing antimicrobial peptides and by controlling mucosal barrier integrity. Prominent sources of

IL-22 are the type 3 ILCs expressing the NK-cell receptor Nkp46, and Th cells expressing IL-22 either exclusively (Th22) or in combination with IL-17. Although IL-22 seems to mediate protection from oxazolone and DSS colitis, it can act as a proinflammatory cytokine in models of skin inflammation [100–102]. Increased numbers of cells expressing IL-22 have been found in the bloodstream and bronchial mucosa of patients with asthma [103], but it is unclear whether the source of this increased IL-22 are ILC3 cells, Th22 cells, or Th17 cells [104, 105]. IL-22 has the potential to promote smooth muscle cell proliferation, which could be important in controlling the BHR that is typical of asthma. In mouse models of asthma, IL-22 appears to have a dual (pro- and anti-inflammatory) role, and studies in IL-22-deficient mice have revealed conflicting results in this regard [104, 106]. Neutralization of IL-22 during sensitization to OVA in an OVA-induced model of asthma in mice severely hampered the development of all asthma features. Conversely, neutralization of IL-22 during allergen challenge increased inflammation, consistent with the potential of IL-22 to enforce mucosal barrier function, and reduce the production of epithelial pro-Th2 cytokines such as IL-25, and the subsequent production of ILC2-derived IL-13 [104–106]. Exactly how IL-22 exerts its anti-inflammatory effects in asthma is still unclear. Administration of rIL-22 to the lungs of mice has the potential to suppress the production of epithelial proTh2 cytokines such as IL-25 [105]. In human bronchial epithelial cells, IL-22 also inhibits the proinflammatory effects of IFN- γ on chemokine secretion [107]. Using an IL-22 overexpression system from a plasmid in mice exposed to OVA, it was also shown that IL-22 can suppress development of allergy, in a process requiring IL-10 [108]. DCs appear to be important regulators of the bioactivity of IL-22 as, in the gut, activated DCs produce the soluble IL-22R protein IL22BP that may play a role in the control of mucosal regeneration [109]. It is not yet clear if lung DCs also regulate the bioactivity of IL-22 during allergen challenge. However, in a chronic model of fungal-induced asthma, IL-22 was shown to be mainly proinflammatory [110].

Role of IL-9-producing Th cells in asthma

Over the past few years, IL-9-producing CD4⁺ T (Th9) cells have been identified as a subset distinct from the classical Th2 cells, with Th9 cells requiring the transcription factors IRF4, PU1, STAT6, Smad3, and Notch signaling for development. Th9 cells differentiate in response to IL-4 and TGF- β and are described to promote T-cell proliferation, IgE, and IgG production by B cells, survival and maturation of eosinophils, and mastocytosis [111–115]. Studies in asthmatic patients have also shown elevated levels of IL-9 in the lungs after allergen challenge; this IL-9 was also demonstrated to be localized to the lymphocyte population in the BAL [116]. Initial mouse studies using transgenic lung-specific overexpression of IL-9 also showed increased airway inflammation, goblet cells metaplasia, and BHR, which were reduced when blocking IL-9 function [117, 118]. Consistent with this observation, later studies using models in which Th9 cells were adoptively trans-

ferred showed that these cells can induce allergic airway inflammation, and that this induction can be reversed by neutralization of IL-9 [112]. IL-9 is also made by ILC2s and boosts production of IL-5 and IL-13, which may in turn amplify Th2-associated inflammation [23]. In a model of chronic *Aspergillus*-induced asthma, IL-9 neutralization suppressed the salient features of disease [119].

Regulatory T cells in asthma

As for any chronic mucosal disorder, it has been proposed that asthma might result from a (functional or absolute) deficiency in natural or induced regulatory T (Treg) cells, either through genetic predisposition, or environmental influences on homeostasis in the immune system. Studies using either the model antigen OVA or mice lacking the intronic *Foxp3* enhancer CNS1 have shown that tolerance mediated by induced Foxp3⁺ Treg (iTreg) cells is the usual outcome after inhalation of harmless antigens [120–123]. Just like natural Treg (nTreg) cells, the iTreg cells found in the airways of mice with asthma highly express high levels of neuropilin-1, whereas iTreg cells in the LNs draining the lung of asthmatics remained neuropilin-1 low [124]. Adoptive transfer studies in mice have revealed that IL-10-producing Treg cells are able to suppress all salient features of asthma, including BHR [125, 126]. Treg cells suppress features of asthma by suppressing the activation of airway DCs (through IL-10 and TGF- β) [127], by reducing (lymph-)angiogenesis [128], and by altering the composition of the gut microbiota. IL-35 is made by ICOS⁺ Foxp3⁺Treg cells and has the potential to suppress the BHR induced by IL-17 in mice [129].

In humans, systemic T-cell responses to allergens in healthy individuals are dominated by TGF- β and/or IL-10. Asthmatic children have reductions in the numbers of pulmonary Foxp3⁺ Treg cells, whereas the number of Treg cells inside the allergen-challenged adult lung is clearly enhanced. This suggests that the function of Treg cells might be suppressed in adults with asthma [130, 131]. TNF- α , IL-6, and TSLP are all overproduced in asthmatic airways and could be responsible for inhibiting the function of Treg cells [132].

The exact mechanism by which Treg cells are induced and recruited to the lungs of asthmatic patients and mouse models of asthma is being intensely studied. Initially, it was shown that DCs expressing ICOS-L and IL-10 were critical for inducing iTreg cells [133, 134]. It was also proposed that plasmacytoid DCs are necessary for Treg-cell formation and/or expansion in the lungs [39, 135]. Recently, Siglec-F⁺ alveolar macrophages were found to be the major APC driving the differentiation of Foxp3⁺ Treg cells in the lungs of mice following allergen inhalation, in a process requiring TGF- β and the retinal dehydrogenases, RALDH-1 and RALDH-2 [136]. The means by which Treg cells become attracted to the allergically inflamed lungs and LNs of mice involves the CCR4 and CCR7 receptor, respectively [137]. The main source of the CCR4 ligands, CCL17 and CCL22, is the CD103⁺ cDC subset of the lungs [34] and targeting antigens to these sDCs using a

Ag-conjugated CD103 mAb has been shown to lead to the expansion and/or accumulation of Treg cells in the lungs [138].

The exact contribution of the intestinal (or pulmonary) microbiota to the induction of Treg cells in the gut and/or lungs is another topic of great interest. Several experiments have now shown that germ-free mice or mice treated with broad-spectrum antibiotics at a very young age have increased features of allergic disease, including increased numbers of basophils and NKT cells [139–143]. These treatments also affect the lung microbiota, but we do not understand the full impact of this on asthma at present [144]. It is possible that the airway microbiota also regulate the threshold for epithelial and immune cell TLR activation, just as the gut microbiota does in colonic epithelium.

Therapeutic implications

Given the clear evidence for IL-4 and/or IL-13 in mouse models of allergic disease, and the presence of Th2 cytokines in patients with asthma, several clinical trials with inhibitors of these cytokines have been launched. A humanized anti-IL-4 neutralizing antibody (pascolizumab) showed promising results in human-derived cell lines and monkeys [145]. However, IL-4-specific antagonists (the IL-4 variant pitrakinra) used in clinical trials have failed to show convincing clinical results [146]. For IL-13, several neutralizing antibodies have been developed (IMA-638, AMG317 (lebrikizumab), and CAT-354), but trials are still in their infancy. Recently, lebrikizumab was shown to improve lung function after 12 weeks of treatment of symptomatic asthmatic patients on standard therapy, particularly when the serum levels of periostin were high [147]. CAT-354 has recently been shown to be safe for use in humans in a phase I clinical trial but its real clinical efficacy remains to be proven [148]. Over the past few years, some evidence suggests that the most effective approaches may be combination therapies interfering with several cytokines and pathways involved in asthma pathogenesis, since anti-IL-4 treatment alone appears to be ineffective and similarly antagonizing IL-13 in mice requires additional suppression of eosinophilic inflammation [149]. IL-4 and IL-13 both use the IL-4R α chain, and blocking this receptor has been developed as a therapeutic strategy. A human monoclonal anti-IL-4R α antibody (AMG317) was developed but showed no clinical efficacy [150], whereas another fully humanized anti-IL-4R α antibody (Dupilumab REGN668) showed clinical efficacy in patients with high peripheral blood eosinophilia upon tapering of inhaled steroids and bronchodilators [151]. Initial proof of concept studies in human asthmatics with anti-IL-5-specific antibody therapies, such as mepolizumab and reslizumab, showed an effective reduction of eosinophil numbers in the blood and sputum of both mild and severe asthmatics, but late allergen responses and BHR were not improved [152, 153]. However, improved efficacy was noticed in specific subgroups of patients with frequent asthma exacerbation and in these patients mepolizumab treatment significantly reduced blood and sputum eosinophil levels and allowed lower corticosteroid

doses to be used to control the inflammation [12, 13]. It seems, however, that for the majority of asthmatic patients, the anti-IL-5 treatment will need to be administered in combination with other therapies that suppress asthma features through other mechanisms. Results of clinical trials targeting the IL-5R α subunit to obtain long-term depletion of eosinophils and basophils are eagerly awaited [154].

Currently, clinical data on anti-IL-9 therapeutics are modest and larger clinical trials are eagerly awaited to conclude whether this form of therapy can be used in the treatment of asthma [155]. Similarly, studies on the neutralization of IL-17 and/or IL-23 and the effect of such neutralization on asthma still need to be reported in humans.

Could ILC2s constitute a therapeutic target? Certainly, given the character of the ROR α nuclear receptor, it might be a target amenable to modification by selective antagonists. Also the precise contribution of ILC2s to asthma pathogenesis in human asthma or in mice with a fully functional adaptive immune system has not been thoroughly explored, as strategies to selectively deplete these cells without affecting other cells of the innate and adaptive immune system have not yet been developed.

Perhaps the most promising aspect of novel asthma therapies would be the potential of restoring the dysbalanced immunity by restoring the deficiency in iTreg-cell numbers. Inhaled corticosteroids already increase iTreg cells in asthmatics, and vitamin D analogs could maybe further enhance this effect [156]. Treg-cell expansion could be achieved by using microbial vaccines or products derived from individual microbes such as TLR9 agonists, inactivated *Mycobacterium bovis* or *Mycobacterium vaccae*, *Helicobacter pylori*, or helminth-derived products [157–159]. Alternatively, specific agonistic antibodies such as the agonistic Ab-stimulating TNFRSF25 (DR3) or CD4 agonistic HIVgp120 have been shown to expand Treg-cell numbers greatly and suppress salient features of asthma [160]. Inhaled drugs increasing the expression of Foxp3 (such as chemically modified Foxp3 mRNA or a cell permeable Foxp3 protein) could similarly achieve this desired effect [161, 162]. Finally active allergen immunotherapy has the ultimate goal of restoring dysregulated immunity in asthma and leads to the expansion of Treg cells (reviewed in [163]).

Concluding remarks

The past few years have seen a renewed interest in the regulation of allergic inflammation, driven by the surge in research on the role of barrier epithelial cells and innate immune cells in regulating asthma. A complex picture emerges whereby epithelial sensing of exogenous and endogenous danger signals leads to the activation of airway DCs and other innate immune cells such as ILCs and basophils. DCs drive expansion of a mixed Th-cell response that is still dominated by Th2 cells, but also includes Th17 cells, Th9 cells, and Treg cells, which induce, exacerbate, or limit various aspects of the disease. We need much more study before we can exploit these novel insights to new therapeutic or preventive strategies for asthma.

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Abbreviations: BHR: bronchial hyperreactivity · GCM: goblet cell metaplasia · HDM: house dust mite · ILC2: type 2 innate lymphoid cell · PRR: pattern recognition receptor · TSLP: thymic stromal lymphopoietin

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