



Airway inflammation in asthma: key players beyond the Th2 pathway

Alex KleinJan

Purpose of review

The Th2 pathway starts with the binding of IL-4 to the IL-4 receptor followed by the phosphorylation of signal transducer and activator of transcription 6 and the activation of GATA3. The most important question relates to the sources of IL-4 and IL-4 related inflammation. Which cells other than Th2 cells are responsible for airway inflammation in asthma?

Recent findings

Accumulating data indicate that basophils contribute to endothelium-related IL-4-dependent inflammation. There is also a dendritic cell-related alternative for the induction of Th2 cells via Notch signalling. GATA3 deoxyribozyme improves asthma that is not clearly related to T-cells. The innate immune response in allergy is linked to mast cells, basophils, and the innate lymphoid cell type 2 (ILC2). ILC2s respond to IL-25, IL-33, thymic stromal lymphopoietin, and leukotrienes by producing IL-4, IL-5, and IL-13. In addition to all this inflammatory-cell-driven asthma, increasing evidence has emerged relating to smooth muscle cell activation, the endothelial and epithelial barrier functions, and improvements in the barrier function. The elevation of intracellular cyclic adenosine monophosphate because of the use of phosphodiesterase inhibitors adds to the prevention of epithelial–endothelial leakage, supports airway smooth muscle relaxation, and is immunosuppressive.

Conclusion and summary

IL-4 is the predominant Th2 cell cytokine. Many more cells, including eosinophils, basophils, mast cells, and ILC2, contribute to the production of IL-4 in the airways. Epithelial cells and endothelial cells lose barrier function in the context of allergic airway inflammation, and this could be at least partially remedied by increasing the intracellular cyclic adenosine monophosphate levels through phosphodiesterase inhibition.

Keywords

endothelial cells, epithelial cells, IL-4, innate lymphoid cell type 2, phosphodiesterase

INTRODUCTION

Asthma is generally known as a Th2 disease. It has become increasingly clear that IL-4 has pathophysiological effects in asthma that extend far beyond the Th2 pathway [1,2]. The Th2 pathway is initiated by the binding of IL-4 to the IL-4 receptor, followed by phosphorylation of signal transducer and activator of transcription 6 and the activation of GATA3. This innate immune response significantly contributes to the allergic airway inflammation, which is linked to eosinophils, mast cells, basophils, and innate lymphoid cell type 2 (ILC2) [3].

IL-4: THE KEY Th2 CYTOKINE IS ALSO PRODUCED BY NON-T CELLS

In a passive skin sensitization model, basophils attach to endothelial vasculature [4^{••}]. When they

remain active, they engage in a long-term interaction with the vasculature [4^{••}]. Continuing activation via immunoglobulin E and the high-affinity receptor for IgE (FcεRI) induces the production of IL-4 and leukotrienes and initially, upon cross-linking, these cells produce histamine in the acute situation [4^{••}]. The long-term (>24 h) allergen activation of peripheral blood mononuclear cells indicates that basophils and natural killer cells are

Department of Pulmonary Medicine, Erasmus MC Rotterdam, Rotterdam, The Netherlands

Correspondence to Alex KleinJan, PhD, Department of Pulmonary Medicine, Room Ee2251, Erasmus MC Rotterdam, PO Box 2040, NL 3000 CA Rotterdam, The Netherlands. Tel: +31 10 7043698; fax: +31 10 7044728; e-mail: a.kleinjan@erasmusmc.nl

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KEY POINTS

- IL-4 is a pleiotropic cytokine that supports airway inflammation beyond the Th2 pathway.
- Epithelial-derived cytokines contribute to and amplify airway inflammation.
- Increasing the intracellular cAMP levels supports immune suppression and improves the endothelial and epithelial barrier function.

an important source of IL-4 (see also Fig. 1 for a summary of IL-4 sources and downstream effects) [5]. In T cells, IL-4 is regulated via GATA3: in several human studies, there was no upregulation of GATA3 when IL-4 expression was increased [6]. In basophils, GATA1 plays a key role in IL-4 induction [7]. Moreover, eosinophils constitute another cellular source for IL-4 [8,9]. The production of IL-4 is important for the induction of adhesion molecules on endothelial cells, promoting and facilitating the development of fibrosis or thrombosis [10]. ILC2, the recently discovered cell that produces IL-5 and IL-13, also produces

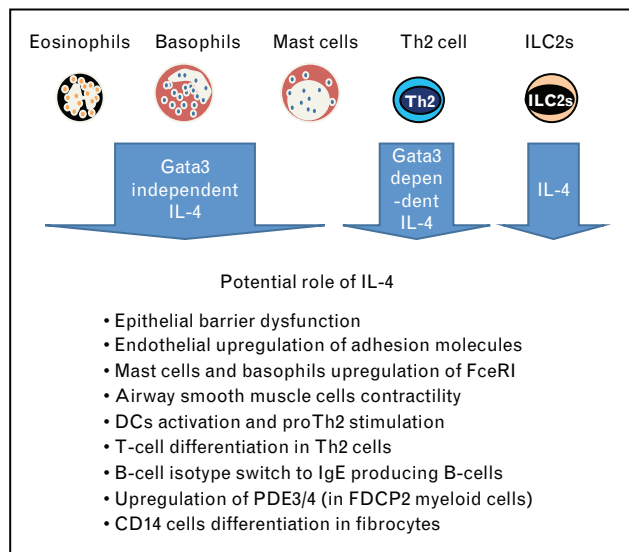


FIGURE 1. Beyond Th2 cells eosinophils, basophils, mast cells, and innate lymphoid cells type 2 are producers of IL-4. IL-4 is a pleiotropic cytokine that act via the IL-4_r on almost all lung cells with the consequence of remodelling of epithelium and lamina propria including profibrotic activity and support of smooth muscle cell contractility. IL-4 act on vasculature by inducing adhesion molecules on endothelial cells to support extravasation of inflammatory cells. IL-4 supports the expression of FcεRI on mast cells and basophils. IL-4 induces the expression of the cyclic adenosine monophosphate specific phosphodiesterase 3 and 4 in factor-dependent cell progenitor (FDCP) 2 cells.

IL-4 in inflammatory conditions, such as in the presence of leukotriene D4 [11,12].

The direct effect of basophil derived IL-4 can be understood in the light of the induction of adhesion molecules like intercellular adhesion molecule-1, vascular cell adhesion molecule (VCAM), and E-selectin [4[¶]]. In allergen challenge studies in humans, it is remarkable that the induction of adhesion molecules is a generalized phenomenon seen in both the upper and the lower airways [13]. Nasal challenge with a relevant allergen induces a rapid accumulation of inflammatory cells, including basophils and eosinophils, within 1 h in the nasal mucosa of allergic rhinitis patients [14]. IL-4, and also IL-13, cause barrier dysfunction in airway epithelial cells [15[¶]]. There are also dendritic cell (DC)-related alternatives for the induction of Th2 cells via Notch signalling.

NOTCH AS AN ALTERNATIVE INDUCER OF Th2 CELLS INDEPENDENTLY OF IL-4

The Notch pathway acts as an alternative pathway for the induction of Th2 cells. In-vitro data from Amsen *et al.* [16] suggest that the Notch pathway, including the Notch ligand Jagged, from the DCs or antigen-presenting cells acts as an important pathway, ultimately inducing T-helper cells that express GATA3 and its corresponding Th2 cytokines, IL-4, IL-5, and IL-13. The Notch pathway is not only T-cell-dependent: the ligands of the Notch pathway expressed by antigen-presenting cells and structural cells also contribute significantly to the interaction of DCs and T cells involving the Notch pathway [16,17]. In populations of activated DCs, Notch ligands express d-like ligand-4 on inflammatory DCs and play an important role in eliciting allogeneic T-cell responses [18]. Moreover, in-vitro granulocyte-macrophage colony-stimulating factor-cultured and LPS-stimulated monocyte-derived DCs express higher levels of the costimulatory molecules Jagged1 and Jagged2 [19].

GLUCOCORTICOID-RESPONSIVE ELEMENT IMPORTANT FOR EFFECTIVE TREATMENT

Glucocorticoid responsive element is located on the side of the DNA where glucocorticoid receptor binds and it supports anti-inflammatory gene transcription, and prevents the forming of proinflammatory gene transcription. In general, Th2 cytokines are affected by corticosteroids [20]. In contrast, cytokine-like IL-8 and IL-33 do not seem to be sensitive to corticosteroids [21].

These underlying mechanisms result in a population of patients that does not benefit from

corticosteroids or only to a limited extent. Asthma and chronic obstructive pulmonary disease (COPD) cover a broad spectrum of lung diseases with asthma at one end and COPD at the other [22]. Patients with neutrophilic asthma are presumably more steroid-resistant and this suspicion is supported by the fact that IL-8 has no glucocorticoid responsive element. mRNA research of the upper airways has confirmed that IL-8 is unaffected and that RANTES is reduced during steroid therapy [23]. It has also been found that IL-17 recruits neutrophils by promoting the release of IL-8 from airway epithelial cells, and that this may be the link between T lymphocytes and granulocytes within the asthmatic airway [24,25].

EPITHELIAL-DERIVED ALARMIN 'PROASTHMA': IL-25, IL-33, AND THYMIC STROMAL LYMPHOPOIETIN

Epithelial cells express a broad range of pathogen-associated molecular patterns and damage-associated molecular patterns. Stimulation of these molecules by allergens has the consequence of cytokine production and release that can activate the innate immune system. Important chemokines are the so-called alarmins [26], including cytokines like IL-33, thymic stromal lymphopoietin (TSLP), and other members of the IL-1 family, including IL-1 α , IL-1 β [27]. In asthma patients, epithelial-derived IL-25 and IL-33 cytokines are responsible for the recruitment of ILC2 and also of basophils and mast cells. The main point is that, if cells express IL-33 receptor (T1/ST2), they may be recruited by this cytokine. The cells that express T1/ST2 are ILC2s, eosinophils, basophils, mast cells, Th2 cells, and DCs (see Fig. 2). IL-33 is a cytokine responsible for the recruitment and/or activation of ILC2s, basophils, and mast cells. TSLP acts on cells that express the TSLP receptor which has been shared with the IL-7 receptor. Targeting TSLP improves allergen-induced bronchoconstriction in asthma [28]. IL-25 is positively correlated with the severity of asthma characteristics including pulmonary parameters (forced expiratory volume in 1 s and methacholine provocation PD20), tissue (sputum and bronchial mucosa) and blood eosinophilia, basement membrane thickness, and immunoglobulin E levels [29]. Respiratory viruses are the most common triggers of exacerbation in asthma: IL-25 is induced and increased in rhinovirus infection [30]. IL-25 at least contributes to the link between allergy, viral infection, and asthma exacerbation by boosting the innate immune response [31]. IL-33 is produced by and is available as stored protein in epithelial cells and endothelial cells. The release of IL-33 in epithelial cells results from damage to and the

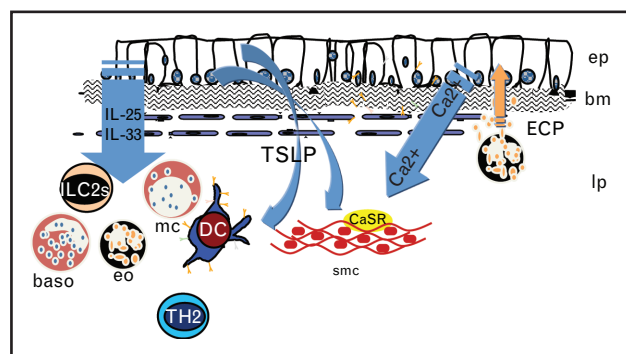


FIGURE 2. Epithelial cells and endothelial cells produce alarmins IL-25, IL-33, and thymic stromal lymphopoietin. These cytokines have the consequence of recruiting and activation of ILC-2, eosinophils, and basophils. Thymic stromal lymphopoietin also acts on smooth muscle cells, eosinophilic cationic proteins, and other polycations act on epithelial cells by elevated extracellular calcium with the consequence of activation of calcium-sensing receptor, leading to intracellular calcium mobilization, cyclic adenosine monophosphate breakdown, and P38 phosphorylation in airway smooth muscle cells.

activation of epithelial cells via their Toll-like receptors. Children with severe asthma and fungal sensitization have been found to have high IL-33 levels and they are associated with corticosteroids-insensitivity [32]. The activation of epithelial cells by fungal proteases induces the release of epithelial IL-33 [33]. ILC2s are cells that play a pivotal role in ongoing inflammation and they could be an even more important source of IL-5 and IL-13 than Th2 cells [34]. ILC2s are antigen-independent and within the airways they are mainly activated by IL-33, but also by IL-25 and TSLP either cumulatively or synergistically. TSLP has also been implicated in alpha smooth muscle cells, supporting airway remodelling and broncho-constriction [35]. Basophils are also recruited and activated via IL-33. Proteases from allergens contribute to the production of IL-33 by epithelial cells and they recruit and activate ILC2s, eosinophils, and basophils. In an IL-33 or papain protease model for allergic inflammation, mast cells were responsible for suppressing airway inflammation via a Mast cell-promoted increase in regulatory T-cell (Treg) numbers [36]. Moreover, it has also been suggested that ILC2s play a role in Treg accumulation in an inducible costimulator (ICOS)-ICOS ligand-dependent mechanism [37]. Both cell types (mast cells and ILC2s) may have a dual function: the stimulation of allergic inflammation during the onset and exacerbation of asthma through the production of Th2 cytokines and raising Treg numbers during the resolution of the airway inflammation.

The potentiation of IL-25 and IL-33 supports allergic airway inflammation and remodelling. TGF β contributes to pulmonary allergic pathology associated with IL-25 [38]. Inflammatory protease relating to neutrophil elastases and cathepsin G can lead to a 10-fold potentiation of IL-33 activity [39]. Mast-cell proteases can cleave IL-33 into a form that is 30 times more potent [40^{***}]. This cleavage could explain why it is only possible to detect IL-33 in low amounts in bronchoalveolar lavage (BAL) fluids, and why it is possible to detect IL-33 in lung homogenates, even though it is not clear whether the IL-33 is bioactive in the latter case.

GATA3 DEOXYRIBOZYME BEYOND THE Th2 PATHWAY

A deoxyribozyme (DNAzyme) that is specific for GATA3 and targets Th2 cells [41], was recently reported to be beneficial in asthma. Krug *et al.* [42^{***}] found an improvement in early- and late-phase asthma response in an allergen provocation model after treatment with DNAzyme. The absence of posttreatment IL-5 induction in the DNAzyme group, which was seen in the placebo group, proves the effectiveness of the therapy, even though it is not clear whether this is because of the targeting of GATA3 in Th2 cells or in ILC2s. Moreover, the levels of tryptase were not increased in the DNAzyme group, while they did increase in the placebo group, suggesting that Th2 cells have an indirect or DNAzyme has a direct effect on mast cell tryptase degranulation [42^{***}]. Basophils express GATA3 and the Fc ϵ RI activation of basophils also increases GATA3 expression. As a result of the activation, IL-4 is also induced [43]. Activated mast cells express GATA3 [44] and could be directly targeted by the DNAzyme, although IL-4 and other Th2 cytokines like IL-13 are found in mast cells, depending on the transcription factor GATA1 and 2 [45]. The detection of the absence of induced levels of tryptase in the DNAzyme study [42^{***}] could be because of less degranulation and a reduction in the release of tryptase by either mast cells or by basophils [14,46].

PHOSPHODIESTERASE IS RESPONSIBLE FOR INTRACELLULAR CYCLIC ADENOSINE MONOPHOSPHATE LEVELS

There is still general interest in phosphodiesterase inhibition, especially of type PDE4B and D, in the field of asthma and COPD. Genome-wide association studies have shown a link between PDE4D and asthma [47,48]. Single nucleotide point mutations have been observed for PDE4D in asthma patients. The biological consequence of these single

nucleotide polymorphisms in PDE4D is the induction of PDE4D activity.

IL-4 induces the expression of PDE3 and PDE4 [49]. Both PDE3 and 4 play a role in tailoring cyclic adenosine monophosphate (cAMP) signalling. Levels of intracellular cAMP are important for the type of immune response and affect the Th1 and Th2 responses.

Low cAMP concentrations in DCs induce and regulate Th2 immunity and allergic asthma. The molecules G α S (which stimulates adenylyl cyclase activity) and G α I (which inhibits membrane adenylyl cyclase activity) regulate cAMP synthesis and the intracellular concentration of cAMP in DCs. As regulators of cAMP, leading either naturally or artificially to low cAMP levels in DCs, low cAMP DCs are proTh2 DCs, resulting in an allergic asthma phenotype, including a Th2 T-cell phenotype [50^{***}].

TARGETING PHOSPHODIESTERASE ISOENZYMES

Inhibitors of PDE3 and 4 are responsible for the increase in intracellular cAMP levels of target cells. Inhibitors of PDE3 act as bronchodilators, whereas the inhibition of PDE4 has also anti-inflammatory effects. The main drawback of the early generation of PDE inhibitors was that they were associated with gastrointestinal (GI) side-effects, nausea, vomiting and emesis, resulting in high study drop-out rates [51]. An inhaled dual PDE3/4 inhibitor, RPL554, has been successfully evaluated in mild asthma and COPD [52,53]. This novel PDE3/4 inhibitor had no GI or other side-effects and had a prolonged bronchodilator effect [52]. Case reports relating to status asthmaticus indicate that the PDE3/4 inhibitor enoximone may be beneficial [54^{***}]. A great benefit of PDE3/4 inhibitors in these several cases is the direct effect as well as long lasting bronchodilator effect [54^{***}]. Enoximone known as a therapy for heart failure has PDE3 and PDE4 inhibitor activities [55]. Several different selective (combined) PDE inhibitors with fewer side-effects are presently under development [56,57].

TARGETING AIRWAY SMOOTH MUSCLE CELLS, ENDOTHELIAL CELLS AND EPITHELIAL CELLS

Polycations, including eosinophilic cationic protein and elevated extracellular calcium, activate the human recombinant and native calcium-sensing receptor (CaSR), leading to intracellular calcium mobilization (see Fig. 2), cAMP breakdown and P38 phosphorylation in airway smooth muscle cells

[58²²]. Both protein and mRNA levels for CaSR were found to be higher in smooth muscle cells in asthmatics than in nonasthmatics. The exposure of airway smooth muscle cells to either TNF- α or IL-13 increases CaSR expression [58²²]. Airway smooth muscle cells from mice lacking CaSR are protected from polycation-induced bronchoconstriction [58²²] and the treatment of wild-type mice with calcilytics prevents methacholine-induced airway hyperreactivity and reduces levels of BAL inflammatory cells and cytokines [58²²]. Calcilytics that blocked the calcium-sensing receptor and that were developed in the past to antagonize the CaSR and treat hypocalcaemia, are currently under investigation in phase I and phase II clinical trials in asthmatics [59²³].

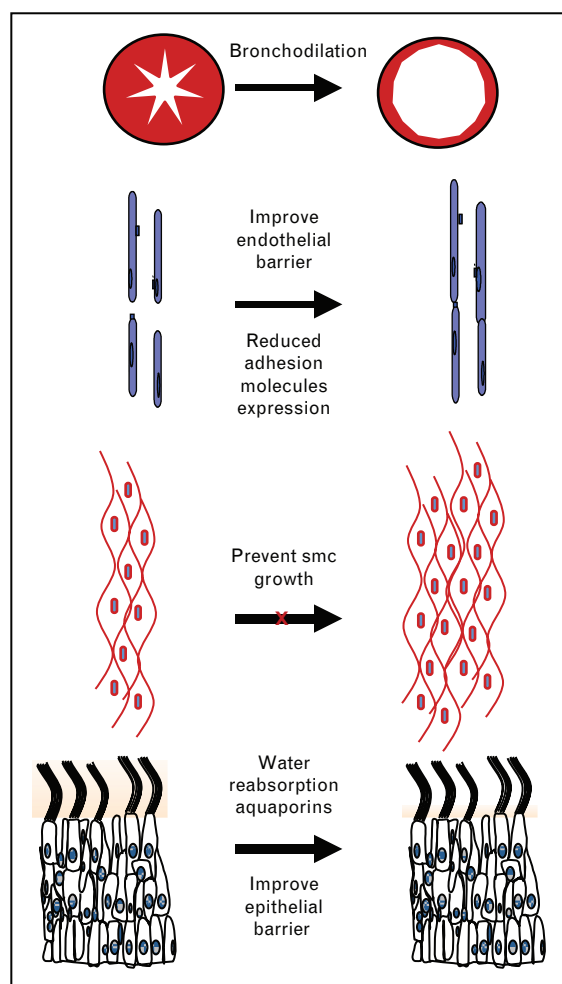


FIGURE 3. Both PDE3 and 4 play a role in tailoring cyclic adenosine monophosphate signalling. PDE3/4 inhibitors are responsible for the increase of intracellular cyclic adenosine monophosphate levels and have anti-inflammatory effects. Effect of PDE3/4 inhibition cause bronchodilation and improve endothelial and epithelial barrier function. PDE, phosphodiesterase.

PDEs are expressed by immune cells and by cells forming the structure of the tissue. PDE inhibitors act effectively on immune cells and on endothelial cells epithelial cells and airway smooth muscle cells (Fig. 3). Endothelial cells express PDE3 and 4, and the blocking of these enzymes results in the inhibition of eosinophil and neutrophil adherence to monolayers of endothelial cells [60,61]. PDE3 and 4 have a synergistic effect on the inhibition of VCAM1 expression and eosinophil adhesion to activated human lung microvascular endothelial cells [60]. The inhibition of PDE3 leads to an increase in cAMP levels, improving the endothelial barrier functions and supporting the cell-cell junction [62²⁴,63]. Moreover, PDE inhibition improves endothelial barrier function as measured in albumin leakage and Evans blue dye extravasation in BAL fluid and or lung tissue in animal models [64,65].

Oxidative stress produced by neutrophils and other cellular sources is believed to play an important role in the pathogenesis of vascular injury and pulmonary oedema. Oxidative stress leads to a time- and dose-dependent increase in hydraulic conductivity and a reduction in the selectivity of an endothelial cell monolayer derived from porcine pulmonary arteries.

Several lines of evidence also indicate that PDE3/cAMP signalling plays a pivotal role in epithelial barrier function and cytokine release. Both cyclic AMP and cyclic guanosine monophosphate signalling modulate membrane water permeability via aquaporin5 expression in pneumocyte type-I cells [66,67]. Experimental lung oedema can be attenuated by selective PDE3 and PDE4 inhibitors [68–71].

OBESITY AND ASTHMA

In a study investigating obesity/asthma and immune-modulating and neuro-hormonal regulation, the obesity-asthma association was not explained by systemic inflammation. Specifically, C-reactive protein, TNF- α , IL-6, neuropeptide Y and adiponectin were independently associated with asthma prevalence. Neuropeptide Y and IL-6 were associated with the IL-4 marker of allergic airway inflammation in asthma and should be further investigated as prognostic markers of asthma outcomes [72].

Bariatric surgery improved small airway function, and reduced systemic inflammation and the number of mast cells within the airways. These effects could explain improvements in asthma control, quality of life, and lung function. In addition to all the other positive effects, bariatric surgery also improves asthma in subjects with morbid obesity [73].

In experimental obese asthmatic mice, restoring normal body weight is an appropriate strategy for reducing TNF- α levels, and controlling inflammation may help to improve asthma severity and control in obesity-related asthma [74]. Moreover, the chitinase-3-like 1 – sirtuin 1 pathway for airway reactivity in obesity seems to be an important pathogenic mediator of allergic airway disease and obesity [75]. Van Huisstede *et al.* [76] found no differences in inflammatory cell counts in bronchial biopsies between obese patients with mild asthma and obese controls. The authors hypothesize that in their cohort asthma is not classically Th2-mediated. Moreover, a subgroup analysis of uncontrolled obese asthmatics had higher levels of the proinflammatory cytokine IL-6 and lower adiponectin levels than obese control patients [76].

CONCLUSION

Asthma is generally known as a Th2 disease. It has become increasingly clear that IL-4 has pathophysiological effects in asthma that extend far beyond Th2 pathway. Moreover, IL-4r may lead to changes being induced through cAMP-dependent pathways. Epithelial-derived alarmins link innate cells and the immune system. The epithelium is one of the most important therapeutic targets of the future for the prevention of airway inflammation remodelling and dysfunction.

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Conflicts of interest

There are no conflicts of interest.

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