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Proinflammatory Pathways in the Pathogenesis of Asthma

R. Stokes Peebles Jr, M.D.^{*,†} and Mark A. Aronica, M.D.[§]

^{*} Division of Allergy, Pulmonary and Critical Care Medicine, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

[†] Department of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

[§] Department of Pathobiology, Cleveland Clinic Lerner College of Medicine of CWRU, Cleveland, Ohio

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The most recent Guidelines for the Diagnosis and Management of Asthma from the National Asthma Education and Prevention Program define the disease thusly: “Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role: in particular, mast cells, eosinophils, neutrophils (especially in sudden onset, fatal exacerbations, occupational asthma, and patients who smoke), T lymphocytes, macrophages, and epithelial cells.”¹

The idea of asthma as a disease of inflammation has gone in and out of style over the years. Aretaeus the Cappadocian (120–180 A.D) described asthma as an inflammation of the lungs, though his understanding of inflammation was vastly different than it is today. Henry Hyde Salter (1823–1871) was the first to describe eosinophils in the sputum of asthmatics, this was closely followed by the description of Charcot-Leyden crystals. During the dawn of modern medicine Sir William Osler published in his textbook of medicine “all authors agree that there is, (in asthma) a strong neurotic element”. It was not until his colleague Robert Cooke took over as editor in 1932 that the text was changed, “In all its types asthma is fundamentally the expression of an allergic reaction.” In the early 1960’s asthma was thought to be a disease of the smooth muscle. In the 1980’s human data returned the focus of asthma to inflammation. The discovery and classification of Th1 and Th2 cells in mice by

corresponding author: Stokes Peebles M.D., Division of Allergy, Pulmonary and Critical Care Medicine, Vanderbilt University School of Medicine, T-1218 MCN, VUMC, 1161 21st Ave South, Nashville, TN 37232-2650, USA, Telephone: 615-343-3412; fax: 615-343-7448, stokes.peebles@vanderbilt.edu.

Mark A. Aronica M.D., Respiratory Institute, Department of Inflammation and Immunity, Cleveland Clinic, 9500 Euclid Ave, NB2-85, Cleveland, Ohio 44195, Telephone: 216-444-6945, aronicm@ccf.org

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Mosmann et al in 1986,² laid the foundation for the classification of asthma as a predominately “Th2 disease”. The discoveries over the subsequent 30 years have shown us that a simple classification of asthma is no longer feasible. Furthermore, the contribution of the importance of research using mice in the discovery of the elements of the allergic inflammatory response cannot be overstated. Many of the cytokines that we now know are critical to the allergic response in humans were first described in mice, initially by discovering their presence in allergic inflammatory response, then by antagonizing those cytokines to determine their specific effect on allergic inflammation, in addition to their contribution on the physiology and pathology of the asthma phenotype.

The link between asthma and inflammation is clear, what has been less clear but has been coming into focus is the heterogeneity and impact of the various inflammatory pathways and the role they play in the currently described asthma phenotypes. Therefore, in this article we will define the known proinflammatory pathways involved in allergic airway inflammation in both mice and humans, and briefly describe therapies that antagonize these pathways and their effectiveness in human asthma. We will also identify elements of these pathways for which there are not currently available therapies, but that could be a future target for asthma treatment.

For the last 50 years, the adaptive immune response has been considered the linchpin of allergic inflammation. While innate immune cells such as mast cells, eosinophils, and basophils were known to have a central role in allergic inflammation, they were dependent upon products produced by B and T lymphocytes to be activated. This adaptive immunity-centric view of the allergic world changed with the first reports of group 2 innate lymphoid cells (ILC2) in 2010.³⁻⁵ With the discovery of ILC2, there was a paradigm shift in that innate cells were recognized to have the ability to produce allergic proinflammatory mediators without the need or assistance of adaptive T and B cell products. In this review, we will start by detailing the mediators involved in activation of the recently discovered innate immunity mediated ILC2-centric pathway. Next, we will focus on specific aspects of the adaptive immune response that do not overlap with the ILC2 response. Finally, we will review lipid mediators in the prostaglandin and leukotriene pathways that regulate allergic inflammatory responses.

INNATE IMMUNITY-MEDIATED ALLERGIC RESPONSE

ILC2

ILC2 is an innate lymphoid subset that is present in tissues where the host interacts with the environment to direct immune responses against pathogens, especially helminths (Figure 1). These tissues in which ILC2 are embedded are predominantly mucosal surfaces of the respiratory and gastrointestinal tracts, in addition to the skin. ILC2 are lineage negative (Lin⁻) in that they do not express markers for T cells, B cells, dendritic cells, macrophages, or granulocytes.⁶ ILC2 have many features of lymphocytes, but lack rearranged antigen receptors, and instead of responding to specific antigens, these cells are activated by soluble mediators that include IL-33, IL-25, and thymic stromal lymphopoietin (TSLP). IL-33, IL-25, and TSLP are all expressed by epithelial cells in response to proteases.⁶ *In vivo* experiments reveal that lung ILC2 have a critical role in rapid inflammation in response to

protease exposure.⁶ Proteases are important constituents of many allergens, such as *Alternaria* and dust mites (Figure 1).⁷ The inflammatory response created by helminth infections has many features of allergic inflammation, as will be discussed later, and is related to the high protease activities of these organisms.⁷ Proteases disrupt mucosal integrity by digesting cell adhesion molecules and also act on protease-activated receptors to activate epithelial cells.⁶ ILC2 produce a cytokine profile that is very similar to CD4 Th2 cells, such as IL-4, IL-5, IL-9, and IL-13; however, the amount of these cytokines is much greater than is produced by Th2 cells. ILC2 indirectly activated by allergens infiltrate the lung and are a major innate source of IL-13.⁸ There is very strong evidence suggesting ILC2 may be critical in the genesis and propagation of allergic responses.^{9–11} Therefore, we will first focus on the cytokines that activate ILC2, before turning our attention to those made by ILC2.

IL-33

IL-33 is predominantly expressed by tissue cell types, including epithelial cells, fibroblasts, and endothelial cells, while its expression may be induced in immune cells such as mast cells and dendritic cells.¹² IL-33 expression is immediately upregulated in the lung from the first day of life and this is closely followed by a wave of IL-13-producing ILC2s. The arrival of lung ILC2 coincides with the appearance of alveolar macrophages (AMs) and their early polarization to an IL-13-dependent anti-inflammatory M2 phenotype.¹³ In mice, IL-33 is predominantly expressed in the lung by alveolar type II pneumocytes, while human lung IL-33 is expressed by bronchial epithelial cells. IL-33 has two major domains, an N-terminal nuclear domain and an IL-1-like domain, and these domains connect by a central domain. The N-terminal domain is essential for nuclear translocation and chromatin association of IL-33.¹² The IL-1-like domain is key to the binding to the IL-33 receptor ST2. Alternative splicing of ST2 produces two different isoforms, a long, transmembrane signaling form of the receptor named ST2L, and a soluble form that lacks transmembrane and intracellular domains called sST2. sST2 is currently believed to be solely a decoy receptor that blocks IL-33 signaling. IL-1R accessory protein (IL-1RAcP) is a co-receptor for IL-33 signaling. ST2 is primarily expressed by immune cells, including ILC2, CD4 T cells, CD8 T cells, follicular T cells, regulatory T cells (Treg), mast cells, macrophages, eosinophils, DCs, basophils, NK cells, and NKT cells. By binding to ST2, IL-33 stimulates receptor-bearing cells to produce and secrete cytokines and growth factors that promote local and systemic immunity. Multiple genome wide association studies (GWAS) revealed that IL-33 and ST2 are significantly associated with asthma.^{14–16} Not only does IL-33 have an important role in Th2-type cytokine production by ILC2 in the innate allergic immune response, but IL-33 also induces antigen-specific IL-5⁺ CD4 T cells and promotes allergen-induced inflammation independent of IL-4.¹⁷

IL-33 is rapidly released into the airway following allergen exposure and elevated levels in BAL fluid occur within one hour.¹² The current concept is that IL-33 exits the cell that produces it by two mechanisms, passive release and active secretion.¹⁸ Passive release of full length IL-33 from the nucleus occurs when the cell is damaged and undergoes necrosis, and in this situation, IL-33 is considered as a damage-associated molecular pattern that activates the immune response as a result of cellular injury. Active secretion of IL-33 occurs when

cells encounter factors that either cause cellular stress or minor repairable injury. For instance, in normal bronchial airway epithelial cells (NHBE), allergens such as *Alternaria* or cockroach induced the translocation of IL-33 from the nucleus to the cytoplasm, followed by extracellular release of the cytokine without apparent cell death.¹⁹ The signaling pathways that are responsible for active IL-33 secretion are not known, however, several elements have been described. Fungal allergen-induced IL-33 release from NHBE cells involved extracellular accumulation of ATP, autocrine and paracrine P2 purinergic receptor activation, and resultant increase in intracellular Ca^{2+} .²⁰ Recently, activation of NADPH oxidase dual oxidase 1 (DUOX1) resulting from the engagement of P2 purinergic receptors was identified as a mechanism for IL-33 release by airway epithelial cells stimulated with *Alternaria* or house dust mite.²¹

Airway exposure of naive mice to a clinically relevant ubiquitous fungal allergen, *Alternaria alternata*, increases bronchoalveolar lavage (BAL) levels of IL-33, followed by IL-5 and IL-13 production and airway eosinophilia without T or B cells.²² This innate type 2 response to the allergen is nearly abolished in mice deficient in ST2, and ILC2 in the lungs are required and sufficient to mediate the response.²² Airway exposure of naive mice to IL-33 results in a rapid production of IL-5 and IL-13 that occurs in less than 12 hours, in addition to marked airway eosinophilia independent of adaptive immunity.²²

Comparison of IL-25 and IL-33 pathway KO mice demonstrates that IL-33 signaling plays a more important *in vivo* role in airways hyperreactivity than IL-25.²³ Additionally, methacholine-induced airway contraction *ex vivo* increases after treatment with IL-33, but not IL-25. This is dependent on expression of ST2 and type 2 cytokines. Confocal studies with IL-13 reporter mice reveal that IL-33 potently induces expansion of IL-13-producing ILC2, correlating with airway contraction. This predominance of IL-33 activity is supported *in vivo* as IL-33 is more rapidly expressed and released in comparison with IL-25. These data reveal that IL-33 plays a critical role in the rapid induction of airway contraction by stimulating the prompt expansion of IL-13-producing ILC2.

Not only does IL-33 stimulate ILC2, but it also promotes the pro-allergic inflammatory properties of CD4 T cells. In a murine model of asthma, ST2 KO mice had attenuated airway inflammation and IL-5 production.¹⁷ Conversely, IL-33 administration induced IL-5-producing T cells and exacerbated allergen-induced airway inflammation in wild type (WT), as well as in IL-4 KO mice. IL-33 is also a chemoattractant for human Th2 cells, but not Th1 cells.²⁴ IL-33, in the presence of antigen, polarized murine and human naive CD4 T cells into a population of T cells that produced mainly IL-5, but not IL-4.

While IL-33 has been comprehensively studied in mouse models of allergic airway inflammation, very few studies have examined its expression in human asthma. ST2L expression was examined in endobronchial brushings and biopsies in patients stratified by asthma severity, as well as by Th2-like biomarkers.²⁵ ST2L expression was significantly increased in severe asthma and significantly associated with multiple indicators of Th2-like inflammation, including blood eosinophils, exhaled nitric oxide, and both epithelial CLCA1 and eotaxin-3 mRNA expression. Multiple single nucleotide polymorphisms in *IL1RL1* were found in relation to dichotomous expression of both ST2L and sST2. sST2 expression

was associated with IFN- γ expression in BAL, while inducing its expression *in vitro* in primary human epithelial cells. In another study, the BAL concentrations of IL-33, TSLP, IL-4, IL-5, IL-13, and IL-12p70, but not IL-25, IL-2, or IFN- γ , were significantly elevated in asthmatics compared with controls.²⁵ The concentrations of IL-33 and TSLP, but not IL-25, significantly correlated inversely with lung function as measured by forced expiratory volume in one second (FEV₁), independently of corticosteroid therapy. These data support a role for IL-33 in the pathogenesis of asthma characterized by persistent airway inflammation and impaired lung function, despite intensive corticosteroid therapy, spotlighting them as potential molecular targets for asthma therapy. Currently, there are no IL-33 antagonists that are currently approved by the FDA; however, there is a major effort by several pharmaceutical companies to develop such molecules given that IL-33 is upstream of Th2 development and ILC2 activation.²⁶ One candidate therapeutic, AMG-282, is an anti-ST2 antibody and is currently in phase I clinical trials for mild allergic asthma and chronic rhinosinusitis (NCT01928368 and NCT02170337). A second candidate therapeutic, ANB020, is being evaluated in phase II trials for the treatment of peanut allergy (NCT02920021).

TSLP

TSLP is a member of the IL-2 cytokine family and is expressed during allergic inflammation by epithelial cells, keratinocytes, and stromal cells.³⁶ The initial prevailing paradigm was that TSLP produced by epithelial cells as a result of exposure to allergen increased DC expression of OX40L to drive Th2 differentiation when antigen was presented by DCs to naïve T cells, initiating an allergic inflammatory response.³⁷ Therefore, determining the mechanisms that regulate TSLP expression and receptor function have been an area of intense investigation over the past decade. However, as TSLP research has progressed, this initial paradigm has become more complex, although the key element that TSLP drives Th2 differentiation and is an important regulator of Th2 responses is widely accepted.³⁶ The TSLP receptor (TSLPR) is a heterodimer consisting of a unique TSLPR subunit and the IL-7R α chain. TSLPR is expressed by a host of hematopoietic cells including T cells, B cells, NK cells, monocytes, basophils, eosinophils, and DCs, as well as by epithelial cells, which are not hematopoietic in origin.³⁶ In TSLPR signaling, TSLPR subunit associated JAK2 interacts with IL-7R α -associated JAK1 to induce STAT5 and STAT1 phosphorylation in CD4 T cells. TSLP has also been shown to act directly on T cells. TSLP, in the presence of CD4 T cell activation through T cell receptor stimulation, promoted the proliferation and differentiation of Th2 cells via induction of *Il4* gene transcription. IL-4 also upregulated the expression of TSLPR on CD4 Th2 cells compared to Th1 and Th17 cells, thus amplifying Th2 responses. The increase in cell surface CD4 T cell TSLPR expression was associated with TSLP's capability to augment the proliferation and survival of activated Th2 cells. B cells express TSLPR and TSLP promotes B cell lymphopoiesis. Innate immune cells also express TSLPR and NKT cells, mast cells, and eosinophils all increase cytokine production in response to TSLP stimulation.³⁶

Mouse models of asthma reveal an important role of TSLP in allergic inflammatory responses. TSLP expression was increased in the lungs of mice with allergen-induced asthma, whereas TSLP receptor KO mice had considerably reduced disease.²⁷ Lung-specific

expression of a *Tslp* transgene induced airway inflammation and hyperreactivity characterized by Th2 cytokines and increased IgE. The lungs of *Tslp*-transgenic mice had massive infiltration of leukocytes, goblet cell hyperplasia and subepithelial fibrosis. TSLP treatment of bone marrow-derived dendritic resulted in their production of the Th2 cell-attracting chemokine CCL17. In a very recent report, TSLP induced the development of pathogenic Th2 cells, which produce increased amounts of the IL-5 and IL-13, while promoting allergic disorders, including asthma.²⁸ TSLP signaling in mouse CD4 T cells initiated transcriptional changes associated with Th2 cell programming. IL-4 signaling amplified and stabilized the genomic response of T cells to TSLP, which increased the frequency of T cells producing IL-4, IL-5, and IL-13. Additionally, TSLP- and IL-4-programmed Th2 cells developed a pathogenic phenotype and produced significantly increased amounts of IL-5 and IL-13 and other proinflammatory cytokines than did Th2 cells stimulated with IL-4 alone. TSLP-mediated Th2 cell induction involved distinct molecular pathways, including activation of the transcription factor STAT5 through the kinase JAK2 and repression of the transcription factor BCL6. In human CD4 T cells, TSLP and IL-4 promoted the generation of Th2 cells that produced greater amounts of IL-5 and IL-13.²⁸ In this report, asthmatic children showed enhancement of such T cell responses in peripheral blood compared to healthy controls.

There is evidence that TSLP may mediate corticosteroid resistant inflammation in the lung, both in mice and in humans. TSLP synthesized during airway inflammation induced ILC2 corticosteroid resistance *in vitro* and *in vivo*, by controlling phosphorylation of STAT5 and expression of Bcl-xL in mouse ILC2 cells.²⁹ Blockade of TSLP with a neutralizing antibody or blocking the TSLP/STAT5 signaling pathway with low molecular-weight STAT5 inhibitors, such as pimozide, restored corticosteroid sensitivity. Dexamethasone inhibited chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes (CRTH2) and Th2 cytokine expression by human blood ILC2s stimulated with IL-25 and IL-33.³⁰ However, it did not do so when ILC2s were stimulated with IL-7 and TSLP, two ligands of IL-7R α . Unlike blood ILC2s, BAL fluid ILC2s from asthmatic patients were resistant to dexamethasone. BAL fluid from asthmatic patients had increased TSLP but not IL-7 levels, and BAL fluid TSLP levels significantly correlated with steroid resistance of ILC2s.³⁰

As opposed to IL-33, there are published clinical trials targeting TSLP in asthma. In a double-blind, placebo-controlled study, 31 patients with mild allergic asthma were treated with three monthly doses an anti-TSLP antibody (tezepelumab) or placebo intravenously and allergen challenges were conducted on days 42 and 84.³¹ Tezepelumab attenuated allergen-induced early and late asthmatic responses and significant decreased blood and sputum eosinophils before and after the allergen challenge, while it also reduced the fraction of exhaled nitric oxide. A more recent phase 2 study of tezepelumab (Reviewed in article by David M. Lang “Immunomodulators and Biologics: Beyond Stepped-Care Therapy”) showed a significantly reduced the rate of asthma exacerbations regardless of blood eosinophil counts at enrollment.³² These two studies provide proof of concept in humans that TSLP has an important role in the pathogenesis of human asthma.

IL-25

IL-25 is expressed by both airway epithelial cells, as well as hematopoietic cells involved in allergic responses, such as Th2 cells, mast cells, basophils, and eosinophils.^{33,34} Similar to IL-33, IL-25 is stored in epithelial cells and released when the cell is exposed to protease containing antigens such as house dust mite.³⁵ IL-25 is a member of the IL-17 family and is also known as IL-17E. The IL-25 receptor is a heterodimer of IL-17RA and IL-17RB and signaling results in activation of NF- κ B, leading to the expression and release of Th2 cytokines, including IL-4, IL-5, and IL-13.^{36,37} The IL-25 heterodimeric receptor complex is expressed on antigen presenting cells, airway smooth muscle, airway epithelial cells, fibroblasts, eosinophils, invariant NKT cells, and ILC2.³⁸ IL-25 activates ILC2 to produce IL-5 and IL-13, but IL-33 is a much more potent and rapid stimulator of ILC2 than IL-25.⁶ Mouse models of allergic inflammation reveal that IL-25 inhibition results in significant decreases in BAL fluid concentrations of IL-5 and IL-13, serum IgE, pulmonary eosinophilia, and abrogated airways responsiveness.^{39,40} IL-25 also had a critical role in recruitment of endothelial progenitor cells to the lung and subsequent neovascularization in an allergen challenge model, suggesting a direct role for IL-25 during angiogenesis *in vivo*. Interestingly, neutralization of IL-25 abrogated the secretion of IL-33 and TSLP, indicating a possible therapeutic strategy for inhibiting all three of these cytokines.⁴⁰

Human studies support a possible role of IL-25 in asthma pathogenesis. In steroid-naïve asthma patients who are stratified based on IL-25 mRNA levels in airway brushings, those with greater IL-25 mRNA levels had a greater allergic phenotype based on allergen skin test positivity and serum IgE levels compared to the IL-25 low subjects or healthy controls.⁴¹ Additionally, the asthma subjects with greater IL-25 mRNA also had greater methacholine responsiveness and evidence of eosinophil activation compared to the IL-25 mRNA low or control subjects. In another study, higher plasma levels of IL-25 and eosinophil receptor expression of IL-17RB were present in patients with allergic asthma compared to healthy controls.⁴² *In vitro* studies revealed that IL-25 augmented methacholine-induced smooth muscle contractility in bronchial rings of subjects with asthma compared to healthy controls.⁴³

Examination of the *in vivo* role of IL-25 in human asthma is incomplete as there has only been one intervention study.⁴⁴ In this trial, a monoclonal antibody targeting IL-17RA, brodalumab, was used in 300 subjects with moderate to severe asthma and there was no therapeutic benefit. However, the IL-17RA subunit is not only a component of the IL-25 receptor, but is also a shared subunit with the IL-17A and IL-17F receptors. Additionally, the subjects in this trial were not phenotyped based on characteristics of IL-25, IL-17A, or IL-17F inflammation. It is interesting that a post-hoc analysis revealed a trend toward improvement with brodalumab treatment in subjects with greater bronchodilator reversibility. Additional data targeting subjects with an IL-25 high phenotype is needed to develop a more complete picture as to the role of IL-25 in asthma pathogenesis.

IL-5

Before being given the name IL-5, this protein had been known as either T-cell-replacing factor (TRF) or eosinophil differentiation factor (EDF).⁴⁵ As EDF, IL-5 stimulated the

production of functional eosinophils in liquid bone marrow cultures and was reported to be specific for the eosinophil lineage in hematopoietic differentiation.^{46,47} As TRF, IL-5 was a potent inducer of IL-2 receptor expression and synergized with IL-2 in the induction of the terminal differentiation of B cells into immunoglobulin secreting cells.⁴⁸ These points are important to remember when considering therapies that target IL-5, as they not only affect eosinophils, but will also impact T and B function. IL-5 signals through a receptor composed of an IL-5 specific α chain and the common β -subunit that maintains the survival and functions of eosinophils and B cells.⁴⁹ In transgenic mice that overexpress IL-5, there is a significant increase in eosinophils and B cells.⁵⁰

Animal models of allergic airway inflammation reveal an important role of IL-5 in pulmonary eosinophilia and airways responsiveness. One time IL-5 challenge of guinea pig airways induced a dose-dependent significant increase in the number of eosinophils, neutrophils, and epithelial cells in BAL fluid 24 hours after administration, and this cell recruitment was inhibited by corticosteroids and ketotifen.⁵¹ In a guinea pig model of acute allergic inflammation induced by ovalbumin sensitization and challenge, anti-IL-5 antibody treatment significantly reduced BAL eosinophils 4 hours after allergen challenge.⁵² In a guinea pig model of chronic ovalbumin challenge, anti-IL-5 treatment not only significantly reduced BAL eosinophilia, but also histamine-induced airway reactivity, while the number of airway neutrophils was not affected.⁵³ Anti-IL-5 treatment also decreased ovalbumin-induced airway responsiveness in mice.⁵⁴ Genetic deficiency of IL-5 receptor α subunit reduced allergen-induced BAL eosinophils and airways responsiveness to acetylcholine compared to WT animals, while there was no difference in BAL IL-5 levels or serum antigen-specific IgE.⁵⁵ The importance of IL-5 in driving airway eosinophilia is further supported by studies revealing that IL-5 overexpression in airway epithelial cells of mice resulted in a dramatic accumulation of peribronchial eosinophilia, bronchial associated lymphoid tissue, goblet cell hyperplasia, focal collagen deposition, and airways responsiveness to methacholine compared to non-transgenic mice.⁵⁶ The importance of eosinophils in IL-5-associated pathology is evident in studies using mice that can generate IL-5, but that have a genetic deletion of eosinophils (PHIL mice). Allergen challenge of PHIL mice revealed that eosinophils were required for pulmonary mucus accumulation and the airway responsiveness associated with asthma.⁵⁷ Allergen-sensitized and challenged PHIL mice had reduced airway levels of Th2 cytokines relative to WT mice and this correlated with a reduced ability to recruit effector T cells to the lung.⁵⁸ In contrast, the combined transfer of antigen-specific T cells and eosinophils into PHIL mice restored the number of effector T cells, airway Th2 immune responses, and asthma-like histopathologic changes. In this model, eosinophils induced the expression of the Th2 chemokines TARC/CCL17 and MDC/CCL22 in the lung after allergen challenge, and neutralization of these chemokines inhibited the recruitment of effector T cells. These results suggest that pulmonary eosinophils are required for the localized recruitment of effector T cells.⁵⁸

These animal studies strongly suggested an important role for IL-5 in human asthma. This was supported by data showing that IL-5 was increased in the serum and bronchial biopsies of patients with asthma.^{59,60} Further, increased serum IL-5 and blood eosinophils were associated with a decrease in FEV₁ during the late phase reactions.⁶¹ Additionally, inhaled IL-5 led to airways responsiveness and sputum eosinophilia in asthma subjects.⁶² While the

first trial utilizing an IL-5 neutralizing antibody, SCH55700, failed to show benefit despite it resulting in a significant reduction in blood eosinophils,⁶³, subsequent studies targeting therapy to the eosinophilic asthma phenotype has supported and confirmed the important role of IL-5 in a this subset of asthma. (Reviewed in article by David M. Lang “Immunomodulators and Biologics: Beyond Stepped-Care Therapy”).

IL-13

IL-13 was first described as a cytokine produced by activated human T lymphocytes that had similar properties to IL-4 in suppressing lipopolysaccharide-induced IL-6, IL-1 β , TNF- α , and IL-8 from human peripheral blood mononuclear cells.⁶⁴ Mapping of the *IL13* gene revealed that it was closely linked to the *IL4* gene on human chromosome 5q 23–31.⁶⁴ The similar biologic effects of IL-4 and IL-13 stem from the fact that they share a common receptor element, IL-4 receptor α (IL-4R α). The IL-13 receptor complex, also known as the IL-4 type 2, is composed of the IL-4R α and IL-13 receptor α 1 (IL-13R α 1). The primary IL-4 receptor is composed of the IL-4R α and the common γ chain (γ c). Signaling through IL-4R α leads to activation and phosphorylation of the transcription factor signal transducer and activator of transcription 6 (STAT6), which when phosphorylated forms a homodimer that can translocate to the nucleus and bind to the GATA-3 promoter, leading to GATA-3 transcription.^{65,66} The IL-13R is expressed on airway smooth muscle cells, endothelial cells, fibroblasts, bronchial epithelial cells, and most leukocytes, including eosinophils, basophils, mast cells and B lymphocytes.^{67–71} It is not expressed on Th1 or Th2 CD4 cells, but is expressed on CD4 Th17 cells.^{72–74} The interaction between IL-17A and IL-13 is very complex. IL-17A enhances IL-13 activity by upregulating IL-13-induced STAT6 activation, while IL-13 inhibits IL-17A intracellular signaling.⁷⁵

Mouse studies reveal an important role of IL-13 in driving the pathognomonic physiologic changes seen in human asthma. *In vivo* neutralization of IL-13, either by antibody or soluble IL-13R, inhibited allergen challenge driven airway responsiveness and mucus metaplasia, but did not affect airway eosinophils, lymphocytes, or serum antigen-specific IgE concentrations.^{76,77} Airway challenge with recombinant IL-13 resulted in induction of airway responsiveness, mucus metaplasia, eosinophilia, and serum IgE.^{76,77} Administration of either IL-13 or IL-4 resulted in an asthma-like phenotype to mice lacking adaptive immune cells by an IL-4R α chain-dependent pathway. Thus, the IL-13 and IL-4 pathways may explain the genetic associations of asthma with both the human 5q31 locus and the IL-4 receptor.⁷⁶

IL-13 has several functional properties that amplify allergic responses. IL-13 and IL-4 selectively induced vascular cell adhesion molecule-1 (VCAM-1) expression in human endothelial cells, suggesting a role in eosinophil tissue accumulation as the VCAM-1 ligand, VLA-4, is expressed on eosinophils.⁷⁸ IL-13 is a B cell stimulating factor and acts at different stages of the B cell maturation pathway as it enhances the expression of CD23/ Fc ϵ R2 and MHC II on resting B cells and stimulates B cell proliferation.⁷⁹ In human B cells, IL-13 induces germline IgE heavy-chain gene transcription, in a similar fashion to IL-4's effect on IgE isotype switching.⁸⁰

IL-13 has been identified in biologic fluids and tissues of patients with asthma, supporting a role for this cytokine in disease pathogenesis. In patients with asthma and rhinitis, there was a significant enhancement of both IL-13 transcripts and secreted proteins in the allergen-challenged BAL compared with the saline-challenged controls, while the expression of IL-13 transcripts was not detected in the BAL of normal subjects challenged with the same dose of allergen. The cellular source of IL-13 mRNA was identified in the mononuclear cell fraction of the allergen-challenged BAL.⁸¹ In a model of endobronchial allergen challenge in humans, there was a highly significant increase in the numbers of eosinophils and both IL-4 and IL-13 after 18 hours in the allergen-exposed segment. In contrast to IL-4, the concentration of IL-13 strongly correlated with the eosinophil numbers found 18 hours after allergen challenge.⁸² IL-13 may be involved in asthma pathogenesis irrespective of the patient's allergic status. Biopsy specimens from subjects with asthma, whether the subjects were atopic or nonatopic, had statistically equivalent quantities of IL-13 mRNA, and these quantities were significantly elevated compared with those in specimens from both the atopic and nonatopic control subjects.⁸³ The quantities of IL-13 mRNA reflected the numbers of activated eosinophils per unit area of submucosa in the biopsy specimens as determined by immunohistochemistry and were statistically equivalent in the atopic and nonatopic subjects with asthma and significantly elevated as compared with those in both the atopic and nonatopic control subjects without asthma. However, there was no correlation between the quantities of IL-13 mRNA and disease severity.⁸³ There is also evidence that IL-13 may contribute to impaired glucocorticoid responsiveness during inflammatory illnesses by decreasing glucocorticoid receptor-binding affinity.⁸⁴

While the presence of IL-13 in BAL or lung tissue is present in patients with asthma, and therefore is associated with disease pathogenesis, recent intervention studies strongly suggest that IL-13 has an important role in disease. A key issue is to phenotype patients to determine which of those subjects may be more responsive to IL-13 antagonists and therefore have a more favorable outcome. Using microarray and polymerase chain reaction analyses of airway epithelial brushings from patients with mild-to-moderate asthma and healthy control subjects, serum periostin, the product of the IL-13-inducible gene POSTN was identified as increased in Th2-high asthma.⁸⁵ Serum periostin was then utilized as a biomarker in a randomized, doubleblind, placebo-controlled trial of the anti-IL-13 antibody lebrikizumab in patients who were inadequately controlled despite inhaled glucocorticoid therapy. At the conclusion of the 12-week trial, there was a significant increase in the mean FEV₁ higher in the lebrikizumab group than in the placebo group.⁸⁶ Among patients in the high-periostin subgroup, the increase from baseline FEV₁ was a statistically significant 8.2 percentage points higher in the lebrikizumab group than in the placebo group. However, in the low-periostin subgroup, the increase from baseline FEV₁ was not different in the lebrikizumab group than in the placebo group. This trial suggested that patients with asthma who had evidence of IL-13-mediated pathobiology of asthma responded to an IL-13 antagonist to a greater degree than those who did not. Recent randomized trials of lebrikizumab have produced mixed results. In the replicate, randomized, double-blind, placebo-controlled trials LUTE and VERSE, treatment with lebrikizumab reduced the rate of asthma exacerbations, with an effect that was more pronounced in the periostin-high patients (60% reduction) than in the periostin-low patients (5% reduction) compared to

placebo.⁸⁷ Lung function was also improved following lebrikizumab treatment, with the greatest increase in FEV₁ in periostin-high patients. However, in replicate phase 3 studies (LAVOLTA I and LAVOLTA II) of patients with uncontrolled asthma despite inhaled corticosteroids and at least one second controller medication, lebrikizumab did not consistently show significant reduction in asthma exacerbations in biomarker-high patients.⁸⁸ In the most recent trial of lebrikizumab in adult patients with mild-to-moderate asthma, lebrikizumab did not significantly improve FEV₁ in mild-to-moderate asthma patients at a dose expected to inhibit the IL-13 pathway.⁸⁹

There have been other strategies to inhibit the IL-13R signaling pathway, most focused on blocking IL-4R α or GATA3, that have included soluble IL-4R α , an agent that prevents assembly of the IL-4R α with either the γ c or IL-13R α 1, or antibodies against IL-4R α . A phase I dose ranging study of a soluble IL-4R α in patients with moderate asthma maintained FEV₁, asthma symptom scores, and β 2-agonist rescue use compared to placebo despite withdrawal of inhaled corticosteroids.⁹⁰ In a follow-up randomized trial of subjects who had asthma exacerbations when inhaled corticosteroids were withdrawn, the soluble IL-4R α prevented decline in FEV₁ and worsening asthma symptom scores compared to placebo, yet there was no difference in withdrawal from the study based on asthma exacerbations.⁹¹ Another strategy to prevent IL-4R α signaling was pitrakinra, a human recombinant protein that prevents assembly of the IL-4R α with either γ c or IL-13R α 1. In two independent randomized, double-blind, placebo-controlled, parallel group phase 2a clinical trials, patients with atopic asthma were treated with pitrakinra or placebo by either subcutaneous injection or nebulization twice daily. Inhaled allergen challenge was performed before and after 4 weeks of treatment.⁹² There was no difference in maximum percentage decrease in FEV₁ between pitrakinra and placebo when the agents were given subcutaneously, but pitrakinra decreased asthma-related adverse events and decreased β 2-agonist rescue events compared to placebo. Pitrakinra nebulization significantly increased FEV₁ compared to placebo, but there were too few asthma-related adverse events to assess the effect of pitrakinra on adverse events.

Monoclonal antibodies against IL-4R α have also been used to determine the effect of this signaling cascade on asthma pathogenesis. In a randomized, controlled, phase 2 study of AMG 317, a fully human monoclonal antibody to IL-4R α , in patients with moderate to severe asthma, the intervention did not demonstrate clinical efficacy across the overall group of patients.⁹³ However, treatment with dupilumab, a fully human monoclonal antibody to IL-4R α was associated with fewer asthma exacerbations when long acting β -agonists and inhaled glucocorticoids were withdrawn in patients with persistent, moderate-to-severe asthma and elevated eosinophil levels.⁹⁴ Dupilumab also improved lung function and reduced levels of Th2-associated inflammatory markers. More recently, dupilumab increased lung function and reduced severe exacerbations in patients with uncontrolled persistent asthma, irrespective of baseline eosinophil count.⁹⁵ These results suggest that blocking IL-13 by itself may not be sufficient in reducing Th2 inflammation, and that the combination of blocking IL-4 and IL-13 signaling through the IL-4R α may be a more robust strategy to inhibit this pathway. These studies also reveal that there is a drug-specific effect, as AMG 317 was not effective while studies with dupilumab have shown it to be efficacious.

Signaling through IL-4R α activates STAT6, which can then translocate to the nucleus to activate GATA3 transcription. SB010, a DNA enzyme that cleaves and inactivates GATA3 messenger RNA was examined in patients with allergic asthma and sputum eosinophilia and who had biphasic early and late phase reactions to inhaled allergen challenge.⁹⁶ The GATA3 antagonist significantly reduced both the early and the late phase reactions compared to placebo, attenuated allergen-induced sputum eosinophilia, decreased serum IL-5 levels, and decreased sputum tryptase levels, while having no impact on the levels of fractional exhaled nitric oxide or methacholine-induced airway responsiveness.

To date, clinical trials modulating the interleukin-13 pathway alone or the combined interleukin-13/interleukin-4 pathways have not revealed serious safety concerns, such as increased risk for infections, malignancy or cardiovascular events.⁹⁷ It will be interesting to follow additional methods developed to antagonize the IL-13 signaling to further define the role of this pathway in asthma pathobiology.

ADAPTIVE IMMUNITY-MEDIATED ALLERGIC RESPONSE

Immune recognition of common environmental antigens is initially regulated by specialized antigen-presenting cells (APCs) such as dendritic cells, macrophages, B lymphocytes and several other cell types.^{48;49} The dendritic cell is the most potent activator of naïve T cells.⁵⁰ Antigens processed by antigen presenting cells through the endocytic pathway are presented as 8–10 amino acid epitopes in MHC class II molecules to CD4 T lymphocytes. CD4 T lymphocytes are generally divided into classes that are characterized by the cytokine array produced by the cell.⁵⁴ Th1 cells are important in the immune response to intracellular pathogens such as viruses and mycobacteria and produce IFN- γ , lymphotoxin, and IL-2.⁵⁵ Th2 cells, as well as mast cells and basophils, produce IL-4, an important factor for B lymphocytes to switch antibody production to the IgE isotype.⁵⁵ Th2 cells additionally make a variety of other proallergic inflammatory cytokines such as IL-5, IL-9, IL-10 and IL-13. As previously mentioned, IL-5 is an important eosinophil regulatory factor and IL-13 is believed to be a central mediator in airway hyperreactivity.^{55;56} Eosinophilic inflammation is one of the hallmarks of the allergic inflammatory response in the airway.⁵⁷ The allergic response to an inhaled antigen is characterized by antigen-specific IgE production by B lymphocytes, whereupon IgE can bind to IgE receptors on tissue mast cells and peripheral blood basophils. When these antigen-specific IgE molecules bound to mast cells and basophils are cross-linked by the specific antigen on antigenic re-exposure, the mast cells and basophils undergo a degranulation process. With degranulation, preformed mediators within the mast cell and basophil such as histamine and tryptase are released, and other mediators such as certain prostaglandins and leukotrienes are generated through the metabolism of arachidonic acid from the cell membrane, as will be detailed in subsequent section.⁵⁷ Although there are many mediators responsible for the full expression of the adaptive allergic response, IL-4 is one of the most important in the adaptive allergic response pathway given its effect on polarizing naïve CD4 lymphocytes to the Th2 pathway and its role in B lymphocyte IgE class switching (Figure 2). Th17 cells will be discussed in this section given their importance in neutrophilic inflammation and a review of T regulatory cells (Treg) is beyond the scope of this article.

IL-4

IL-4 was originally known as B cell growth factor (BCGF), and then B cell stimulatory factor-1 (BSF-1), as it augmented the proliferative response of B cells to anti-IgM.⁹⁸ In 1986, IL-4 was identified as a potent switch factor for B cell IgE synthesis.⁹⁹ *IL4* maps to the human chromosome 5q31 and is 12 kB from the *IL13* gene.¹⁰⁰ IL-4 predominantly signals through the type I IL-4 receptor that is comprised of the IL-4R α and γ c. In lymphoid cells, IL-4R α associated with JAK1 and γ c with JAK3. IL-4 and IL-13 are unique in that they are the only ligands that caused STAT6 phosphorylation.¹⁰¹ IL-4 signaling through the type I IL-4 receptor acts relatively early for naïve CD4 T cells to acquire IL-4-producing capacity and develop into Th2 cells.¹⁰² CD4 Th2 cells are capable of producing IL-4, IL-5 and IL-13, and are critical for the humoral immune response against extracellular pathogens and in the induction of allergic diseases, including asthma. While STAT6 is downstream of IL-4R signaling, enhanced STAT5 signaling also induced Th2 differentiation independent of IL-4 signaling, and although it did not upregulate GATA-3 expression, it did require the presence of GATA-3 for its action. Therefore, there is a critical role for GATA-3 in Th2 cell differentiation (both IL-4 dependent and IL-4 independent) and in inhibiting Th1 differentiation.¹⁰³ In addition to CD4 Th2 cells, other cells that produce IL-4 induce NK T cells, basophils, mast cells, and eosinophils.^{104–109} ILC2 can be induced to produce IL-4 in response to cysteinyl leukotrienes.¹¹⁰

In addition to IL-4 promoting IgE isotype switching and polarization of naïve T cells into Th2 cells, IL-4 also induced expression of VCAM-1, which directed the migration of T cells, monocytes, basophils, and particularly eosinophils to sites of allergic inflammation.^{111,112} IL-4 also induced mucin gene expression, resulting in increased airway mucus production.¹¹³

Allergen challenge studies in mice revealed the importance of IL-4 in the development of the asthma phenotype. Allergen sensitized and challenged C57BL/6 mice resulted in substantially fewer eosinophils in BAL and much less peribronchial inflammation in IL-4 KO mice compared with WT mice.¹¹⁴ These results suggested that IL-4 is a central mediator of allergic airway inflammation, regulating antigen-induced eosinophil recruitment into the airways by a T cell dependent mechanism.¹¹⁴ The relationship between IL-4, IL-5, and airway eosinophils was further defined when CD4 T cells, and not CD8 T cells were necessary to induce eosinophilic inflammation.¹¹⁵ Finally, antibody neutralization of IL-4 during the period of systemic immunization abrogated airway responsiveness, but had little effect on the influx of eosinophils.¹¹⁶ Administration of anti-IL-4 only during the period of the aerosol challenge did not affect the subsequent airway response to acetylcholine. Administration of anti-IL-5 antibodies at levels that suppressed eosinophils to less than 1% of recruited cells had no effect on the subsequent airway responses. These results revealed that IL-4 generated during the period of lymphocyte priming with antigen was critical in generating airway responsiveness to inhaled antigen, while no role for IL-5 or eosinophils in airway responsiveness could be demonstrated.¹¹⁶

Many investigators found IL-4 present in biologic fluids and cells from patients with asthma, supporting a role for this cytokine in the pathogenesis of allergic airway inflammation. In subjects with mild atopic asthma, mRNA expression of IL-4, IL-5, GM-CSF, and IL-2 was

greater in BAL cells compared to normal control subjects, while there was no difference between the two groups in mRNA expression of IFN- γ in BAL cells as assessed by simultaneous in situ hybridization and immunofluorescence.¹¹⁷ This study revealed that atopic asthma was associated with a pattern compatible with predominant activation of the Th2-like T-cell population.¹¹⁷ Others found a distinction in IL-4 expression based on whether the patient had allergic or nonallergic asthma. In one study, increased levels of IL-4 and IL-5 characterized allergic asthmatics, and this elevated IL-4 contributed to the increased IgE levels found in these allergic subjects. In contrast, nonallergic asthmatics had elevated levels of IL-2 and IL-5, with IL-2 contributing to T-cell activation. In both types of asthma, the close correlation of IL-5 levels with eosinophilia suggests that IL-5 is responsible for the characteristic eosinophilia of asthma. Thus, there was evidence of distinct T-cell activation resulting in different spectra of cytokines in allergic and nonallergic asthma.¹¹⁸ Corticosteroids reduced the number of IL-4 expressing cells and eosinophils in BAL fluid, while increasing the number of IFN- γ expressing cells.¹¹⁹ Finally, whole lung allergen challenge increased the number of activated CD4 T cells expressing IL-4 and IL-5 twenty-four hours after the challenge, while there was no evidence of activation of CD8 T cells.¹²⁰ At the 24-hour post challenge time point, there was a significant increase in the number of eosinophils that correlated with the maximal late fall in FEV₁. These results revealed that cytokines produced by activated Th2-type CD4 T cells in the airway contributed to late asthmatic responses by mechanisms that included eosinophil accumulation.¹²⁰

While antagonists targeting IL-4R signaling have been used to determine the contribution of this pathway in asthma pathogenesis, as was reviewed in the IL-13 section, specific IL-4 antagonists have not been used in human asthma trials.

IL-17

IL-17A and IL-17F have been strongly implicated in the neutrophilic inflammation that occurs in a subset of asthma patients.¹²¹ IL-17A and IL-17F regulate neutrophilic influx into tissues by inducing airway epithelial cell and stromal cell production of cytokines such as G-CSF, GM-CSF, and IL-6, in addition to chemokines such as CXCL8, CXCL6, and CXCL1, that promote neutrophil chemotaxis and survival (Figure 3).¹²² Thus, IL-17A and IL-17F do not directly cause neutrophil chemotaxis, but act indirectly by promoting the release of pro-neutrophilic mediators.¹²³ A number of cell types, including CD4 Th17 cells, $\gamma\delta$ T cells, NKT cells, NK cells, ILC3, and mast cells express IL-17A and IL-17F.^{124,125} While some investigators report that neutrophils express IL-17A and IL-17F, others do not.¹²⁶ IL-17A and IL-17F share a common receptor complex, IL-17RA and IL-17RC, and is likely the predominant reason for the common biological function of these two cytokines.¹²⁷ In terms of importance to asthma pathogenesis, the IL-17 receptor is expressed on airway smooth muscle cells, epithelial cells, fibroblasts, macrophages, and endothelial cells. CD4 Th17 cells are a major source of IL-17A and IL-17F. Based on our current understanding, there are three different combinations of cytokines that differentiate naïve CD4 T cells into Th17 cells. These include IL-6 and TGF- β , with additive potentiating effects of IL-1 β and TNF; IL-21 and TGF- β ; and IL-6, IL-1 β , and IL-23.^{128–130} Key transcription factors activated in the Th17 differentiation process include STAT3 and RORC2.¹²⁹

Animal models investigating the impact of Th17 cytokines suggest an important role in asthma pathogenesis. IL-17RA KO mice that are unable to respond to IL-17A or IL-17F, had decreased ovalbumin-induced allergic airway inflammation compared to WT mice.¹³¹ IL-17A protein expression synergized with IL-13 present during allergic airway inflammation to increase airways responsiveness in a complement C5a dependent manner.¹³² Th17 cells mediated steroid-resistant airway inflammation and airway responsiveness in mice.¹³³ These results suggest that IL-17A increases Th2-mediated airway responsiveness and airway inflammation. However, it is important to note that other groups have reported that IL-17A had an anti-inflammatory role in allergic airway inflammation. For instance, instillation of recombinant mouse IL-17A during ovalbumin-challenge decreased AHR, eosinophil infiltration into the airways, and expression of CCL5, CCL11, and CCL17.¹³¹ Further, neutralization of IL-17A during allergic airway inflammation increased airway responsiveness and eosinophil infiltration into the airways.¹³⁴

Most studies investigating the presence of IL-17A in asthma support that this cytokine was increased in the airway cells and BAL fluid. IL-17A was increased in the airways of asthma subjects and induced human bronchial fibroblasts to produce proinflammatory cytokines.¹³⁵ There was increased expression of airway cells expressing IL-17A and IL-17F in patients with mild-moderate asthma compared to healthy controls.¹³⁶ Further, there was increased IL-17 mRNA in the sputum of mild and moderate/severe asthma subjects compared to healthy controls and this was associated with an increase of the percentage of neutrophils to total granulocytes.¹³⁷ *In vitro* studies support a role for IL-17A in asthma pathogenesis. IL-17 enhanced production of eotaxin, an important chemokine in eosinophil migration, by primary airway smooth muscle cells.¹³⁸ IL-17A also increased proliferation of human primary tracheal epithelial cells.¹³⁹ IL-17A induced MUC5AC and MUC5B mucins in primary human tracheobronchial epithelial cells.^{140,141} IL-17A, IL-17F, and IL-22 are associated with increased airway smooth muscle proliferation and migration.^{142,143}

While there is abundant data from *in vivo* mouse experiments, human airway surveys, and *in vitro* studies using human cells, as mentioned earlier, the lone human asthma clinical trial examining IL-17 antagonism with brodalumab did not show clinical improvement.⁴⁴ Recently, the FDA issued a black box warning after 6 patients treated with brodalumab across four clinical trials committed suicide,¹⁴⁴ therefore, there will likely be no further clinical asthma trials for this drug. However, further exploration of the IL-17 pathway antagonism is warranted in appropriately phenotyped patients who have a Th17 signature with neutrophilic inflammation given the association of neutrophilic inflammation with severe disease.

EICOSANOID PATHWAY

Eicosanoids are 20 carbon chain lipids formed from the metabolism of arachidonic acid that have potent activities in a number of biologic properties. In terms of allergic disease, the two pathways that most promote allergic inflammation are prostaglandin (PG) D₂ and the cysteinyl leukotrienes (LT), formed from the cyclooxygenase (COX) and leukotriene pathways, respectively. As shown in figure 4, arachidonic acid is formed by the cytosolic phospholipase A₂ (cPLA₂) cleavage of membrane phospholipids. There are two COX

enzymes. COX-1 is largely constitutively expressed, while COX-2 may be induced by inflammatory stimuli such as lipopolysaccharide (LPS), IL-1, or TNF. Both COX-1 and COX-2 metabolize arachidonic acid into an unstable intermediate, PGH₂, and then tissue specific enzymes and isomerase synthesize the five primary PGs, which are PGD₂, PGE₂, PGF_{2α}, PGI₂ and thromboxane A₂ (TXA₂). LT are generated when the 5-LO pathway metabolizes arachidonic acid.¹⁴⁵ The released arachidonic acid binds to 5-LO activating protein (FLAP) for presentation to 5-LO for oxygenation and the production of LTA₄.¹⁴⁶ LTA₄ can be metabolized by LTA₄ hydrolase into LTB₄, or can be metabolized by LTC₄ synthase (LTC₄S) to LTC₄, which can then sequentially be metabolized to LTD₄ and finally LTE₄. LTC₄, LTD₄, and LTE₄ are known as the cysteinyl LT because of the presence of cysteine in their structure.

PGD₂

PGD₂ is the major mast cell-derived prostanoid and is released in nanogram quantities in these cells in response to IgE-mediated activation.¹⁴⁷ Eosinophils also synthesize PGD₂.¹⁴⁸ The prostanoids signal through distinct seven transmembrane, G-protein coupled receptors (GPCRs). The receptors through which PGD₂ signals are termed DP₁ and DP₂ (Figure 4).¹⁴⁷ DP₁ is expressed on mucus-secreting goblet cells in the nasal and colonic mucosa, nasal serous glands, vascular endothelium, Th2 cells, DCs, basophils, and eosinophils. DP₁ stimulation activates adenylate cyclase, resulting in increased intracellular cAMP and protein kinase A activity. DP₂ is also known as chemoattractant receptor-like molecule expressed on Th2 cells (CRTH2) and is expressed on eosinophils, basophils, and the T cell subsets CD4 Th2 and CD8 Tc2 cells. PGD₂ induces chemotaxis in each of these immune cells in a DP₂-dependent manner. DP₂ is preferentially expressed by T cells expressing IL-4 and IL-13 compared to T cells expressing IFN-γ in the BAL fluid of subjects with asthma.¹⁴⁹ DP₂ signaling in eosinophils augments their release from bone marrow, increases their respiratory burst, stimulates the chemotactic response to other chemokines such as eotaxin, and primes them for degranulation. Further, DP₂ signaling upregulated microvascular permeability, reduction of goblet cells, and constricted coronary arteries. In contrast to DP₁ signaling, activation of DP₂ reduced intracellular cAMP.¹⁴⁷ Therefore, PGD₂ signaling through DP₂, by suppressing cAMP, would be predicted to facilitate allergic inflammation through its effect on chemotaxis and mediator release by effector cells. PGD₂ stimulated human peripheral blood ILC2 to secrete large amounts of IL-13 to the same level produced in response to IL-25 and IL-33, whereas the addition of IL-25 and IL-33 to PGD₂ synergistically increased IL-13 expression by ILC2.¹⁵⁰ In these experiments, PGD₂ increased IL-13 production by ILC2 mainly through activation of DP₂.¹⁵⁰ Others similarly reported that PGD₂ enhanced human ILC2 function.¹⁵¹ DP₂ signaling enhanced ILC2 migration and production of Th2-like cytokines by ILC2. PGD₂ activation through DP₂ increased ILC2 expression of the IL-33 and IL-25 receptor subunits, ST2 and IL-17RA, respectively.¹⁵¹ Cysteinyl LT, particularly LTE₄, enhances the activation of ILC2 by PGD₂.¹⁵² LTE₄ increased Type 2 cytokine production stimulated by PGD₂, IL-25, IL-33, and TSLP, in addition to IL-2-induced increases in IL-33 and IL-25 receptor expression on ILC2.

Inhalation challenge of allergic asthmatic subjects with allergens to which the subjects were sensitized augmented PGD₂ in BAL.¹⁵³ PGD₂ levels in BAL were increased in patients with severe asthma, even at baseline in the absence of allergen challenge.¹⁵⁴ Asthma exacerbations, poor asthma control, and markers of Th2 inflammation were associated with higher PGD₂ levels, hematopoietic PGD synthase, and DP₂.¹⁵⁴ PGD₂ was also increased in the nasal lavage from subjects with allergic rhinitis,¹⁵⁵ in tears from persons experiencing allergic conjunctivitis,¹⁵⁶ and in the fluid obtained from experimentally produced skin blisters in patients with late phase reactions of the skin.¹⁵⁷

There are several published trials of DP₂ antagonists in humans with asthma and other allergic diseases. OC000459 significantly improved quality of life and night-time symptom score and reduced geometric mean sputum eosinophil count compared to pre-treatment baseline, but not placebo.¹⁵⁸ This DP₂ antagonist has also shown some efficacy in adult patients with corticosteroid-dependent or corticosteroid-refractory eosinophilic esophagitis (EoE).¹⁵⁹

The DP₂ antagonist BI 671800 significantly improved nasal symptom scores, decreased nasal eosinophils, reduced nasal IL-4 and eotaxin levels in a dose-related manner in patients with seasonal allergic rhinitis.¹⁶⁰ BI 671800 was also examined in patients with asthma in two separate trials.¹⁶¹ In the first trial, BI 671800 significantly increased FEV₁ greater than the change in FEV₁ seen with placebo; however, there was no significant change in asthma control questionnaire (ACQ). In the second trial, BI 671800 significantly increased FEV₁ compared to placebo and significantly increased the mean ACQ score at a high dose, although this increase was not deemed to be clinically significant.¹⁶¹ In a more recent phase IIa, 12-week, BI 671800 treatment did not result in a statistically significant or clinically meaningful difference in the ACQ scores compared to placebo.¹⁶² The oral DP₂ antagonist fevipiprant was examined in patients with mild-to-moderate uncontrolled allergic asthma.¹⁶³ While there was no benefit with fevipiprant in the entire study population, a subgroup analysis revealed that patients with an FEV₁<70% predicted at baseline had a significant improvement in trough FEV₁ and ACQ7 score compared to placebo. In another study, fevipiprant treated patients had a decrease in the mean sputum eosinophil percentage, and this was significantly greater than the change in sputum eosinophils in the placebo-treated patients.¹⁶⁴ The DP₂ antagonist AZD1981 was examined in adults with asthma and the treatment had no significant effect on morning peak expiratory flow. In another study of patients with uncontrolled asthma despite inhaled corticosteroid therapy AZD1981 significantly increased ACQ-5 scores, but there was no dose-response relationship.¹⁶⁵ Additional studies will be important to confirm the clinical usefulness DP₂ antagonism in asthma, but these trials suggest that PGD₂ is involved in asthma pathogenesis.

Cysteinyl LTs

The major cellular sources of cysteinyl LTs are eosinophils, basophils, mast cells and macrophages, each of which express LTC₄S. LTC₄S expression is sharply upregulated in human mast cells by IL-4-STAT6-dependent transcription, potentially reflecting a mechanism for upregulating cysteinyl LT production in allergic inflammation.¹⁶⁶ Both LTB₄ and LTC₄ are exported by specific respective transporter proteins, members of the multidrug

resistance proteins (MRPs). MRP1 is a specific transmembrane protein that transports LTC₄ to the extracellular space. There are three cysteinyl LT receptors, cysLT₁, cysLT₂, and cysLT₃. CysLT₁ is expressed on airway smooth muscle cells, eosinophils, B cells, mast cells, monocytes, and macrophages.^{167,168} Signaling through cysLT₁ induced bronchoconstriction, mucus secretion, and airway edema. Therapeutic cysLT₁ antagonists include montelukast, pranlukast, and zafirlukast. CysLT₁ expression is upregulated at the transcriptional level by type 2 cytokines, including IL-4 and IL-13, providing an explanation for increased cysLT₁ expression in subjects with allergic diseases. CysLT₂ is expressed on lung macrophages, airway smooth muscle, peripheral blood leukocytes, mast cells, and brain tissue.¹⁶⁹ In mice, cysLT₂ is expressed in the lung on bronchial smooth muscle, alveolar macrophages, conventional dendritic cells, and eosinophils.¹⁷⁰ A cysLT₂ antagonist inhibited multiple antigen challenge-induced increases in eosinophils and mononuclear cells into the lung.¹⁷⁰ Currently, there are no selective cysLT₂ inhibitors in clinical use, thus the function of cysLT₂ is based on animal studies. Studies in cysLT₂ KO mice suggest that signaling through cysLT₂ promotes vascular permeability, inflammation, and tissue fibrosis, but not bronchoconstriction. CysLT₃ was recently discovered and was previously known as GPR99. The predominant ligand for cysLT₃ is LTE₄ and there are no known human cysLT₃ antagonists. Animal studies using cysLT₃ KO mice revealed that these mice had a dose-dependent loss of LTE₄-mediated vascular permeability, but not to LTC₄ or LTD₄, suggesting a preference of cysLT₃ for LTE₄ even when CysLT₁ is present.¹⁷¹ CysLT₃ was detected on lung and nasal epithelial cells in mice.¹⁷² Following either *Alternaria alternata* or LTE₄ airway challenge in mice, cysLT₃ KO mice were protected against profound epithelial cell mucin release and swelling. CysLT₃ KO mice have decreased baseline numbers of goblet cells, suggesting a function of this receptor in regulating epithelial cell homeostasis.¹⁷²

There have been two productive therapeutic strategies to antagonize LTs. The first is to decrease the production of the LTs by inhibiting their synthesis through 5-LO. The second strategy is to reduce LT binding on target tissues via LT receptor antagonists. Inhibiting 5-LO synthesis would have the combined benefit of reducing production of both LTB₄ and the cysteinyl LTs. Theoretically, 5-LO inhibition would blunt cysteinyl LT-induced bronchoconstriction and vascular permeability, as well as LTB₄-mediated neutrophil and eosinophil chemotaxis. Zileuton is the only 5-LO inhibitor approved for the treatment of asthma.¹⁷³ Unfortunately, zileuton has therapeutic shortcomings that has limited its widespread acceptance for asthma treatment. These include a short half-life and the potential for liver toxicity. Thus, 5-LO inhibitor use requires multiple daily doses and liver function testing. An extended release form of zileuton is available that may be prescribed twice daily as opposed to four times a day; however, the total daily dose is the same.

CysLT₁ antagonists include montelukast, pranlukast, and zafirlukast. Most of the clinical trials of examining the therapeutic effect of LTs in asthma and allergic diseases have been in this class of medications and confirmed that cysteinyl LTs contributed a major portion of the bronchospasm that resulted from allergen challenge. Administration of the cysLT₁ antagonist zafirlukast prior to allergen challenge inhibited immediate phase bronchospasm by approximately 80% and reduced the late phase by 50%.^{174,175} CysLT₁ antagonists and 5-LO inhibitors blunted the influx of airway inflammatory cells after segmental allergen

challenge.^{176,177} Exercise-induced bronchospasm (EIB) is consistently blunted by both cysLT1 antagonists and 5-LO inhibitors by approximately 30–60%.^{178–181}

Approximately 10% of adults with asthma will have asthma symptoms and reduction in pulmonary function after ingesting aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs), and this is termed aspirin exacerbated respiratory disease (AERD).¹⁸² The exact mechanisms causing this adverse response are not fully defined; however, there is increased expression of LTC₄ synthase and cysLT2 by mast cells and eosinophils in patients with AERD. It is unknown whether the increase in LTC₄ synthase expression is an underlying mechanism leading to AERD. Treatment with both cysLT1 antagonists and 5-LO inhibitors reduced AERD pulmonary function abnormalities and symptoms, strongly suggesting that cysLTs are involved in AERD pathogenesis. Several studies revealed that there is almost 100% inhibition of aspirin-induced bronchospasm by LT antagonists.^{183–185} 5-LO inhibition decreased aspirin-induced urinary LTE₄ and mast cell produced tryptase in nasal secretions.^{184,186} These results substantiate that LT antagonists impact mast cells and their activation, but the importance of LT generation as a result of mast cell activation in this setting needs further definition.

CONCLUSION

Discovery of proinflammatory pathways involved in asthma pathogenesis is far from complete. Just when it seems that we know all there to know is about asthma inflammation, a major paradigm shift occurs which teaches us that we still have a lot to learn. The two most recent examples of this include the discovery of the Th17 cells in the early 2000s and the recognition of ILC2 earlier this decade. Continued close observation and investigation will lead the field to future discoveries of molecules and cells that are increased in patients with asthma. These targets will be able to be antagonized quickly in both mice and people because of advancements in molecular biology and pharmacology, providing the opportunity to directly test their involvement in the asthma phenotype, with the goal of improving patient care of people with this disease.

References

1. National Heart L, and Blood Institute. Guidelines for the diagnosis and management of asthma (EPR-3). 2007
2. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol.* 1986;136(7):2348–2357. [PubMed: 2419430]
3. Durbin JE, Johnson TR, Durbin RK, et al. The role of IFN in respiratory syncytial virus pathogenesis. *J Immunol.* 2002;168(6):2944–2952. [PubMed: 11884466]
4. Neill DR, Wong SH, Bellosi A, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature.* 2010;464(7293):1367–1370. [PubMed: 20200518]
5. Price AE, Liang HE, Sullivan BM, et al. Systemically dispersed innate IL-13-expressing cells in type 2 immunity. *Proc Natl Acad Sci U S A.* 2010;107(25):11489–11494. [PubMed: 20534524]
6. Halim TY, Krauss RH, Sun AC, Takei F. Lung natural helper cells are a critical source of Th2 celltype cytokines in protease allergen-induced airway inflammation. *Immunity.* 2012;36(3):451–463. [PubMed: 22425247]

7. Phillips C, Coward WR, Pritchard DI, Hewitt CR. Basophils express a type 2 cytokine profile on exposure to proteases from helminths and house dust mites. *J Leukoc Biol.* 2003;73(1):165–171. [PubMed: 12525574]
8. Barlow JL, Bellosi A, Hardman CS, et al. Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity. *J Allergy Clin Immunol.* 2012;129(1):191–198. [PubMed: 22079492]
9. Doherty TA, Khorram N, Chang JE, et al. STAT6 regulates natural helper cell proliferation during lung inflammation initiated by *Alternaria*. *Am J Physiol Lung Cell Mol Physiol.* 2012;303(7):L577–L588. [PubMed: 22865552]
10. Drake LY, Iijima K, Bartemes K, Kita H. Group 2 Innate Lymphoid Cells Promote an Early Antibody Response to a Respiratory Antigen in Mice. *J Immunol.* 2016;197(4):1335–1342. [PubMed: 27421480]
11. McKenzie AN. Type-2 innate lymphoid cells in asthma and allergy. *Ann Am Thorac Soc.* 2014;11 Suppl 5:S263–270. [PubMed: 25525730]
12. Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol.* 2016;16(11):676–689. [PubMed: 27640624]
13. Saluzzo S, Gorki AD, Rana BMJ, et al. First-Breath-Induced Type 2 Pathways Shape the Lung Immune Environment. *Cell Rep.* 2017;18(8):1893–1905. [PubMed: 28228256]
14. Moffatt MF, Gut IG, Demenais F, et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med.* 2010;363(13):1211–1221. [PubMed: 20860503]
15. Savenije OE, Mahachie John JM, Granell R, et al. Association of IL33-IL-1 receptor-like 1 (IL1RL1) pathway polymorphisms with wheezing phenotypes and asthma in childhood. *J Allergy Clin Immunol.* 2014;134(1):170–177. [PubMed: 24568840]
16. Torgerson DG, Ampleford EJ, Chiu GY, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet.* 2011;43(9):887–892. [PubMed: 21804549]
17. Kurowska-Stolarska M, Kewin P, Murphy G, et al. IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol.* 2008;181(7):4780–4790. [PubMed: 18802081]
18. Morita H, Nakae S, Saito H, Matsumoto K. IL-33 in clinical practice: Size matters? *J Allergy Clin Immunol.* 2017;140(2):381–383. [PubMed: 28478050]
19. Drake LY, Kita H. IL-33: biological properties, functions, and roles in airway disease. *Immunol Rev.* 2017;278(1):173–184. [PubMed: 28658560]
20. Kouzaki H, Iijima K, Kobayashi T, O'Grady SM, Kita H. The danger signal, extracellular ATP, is a sensor for an airborne allergen and triggers IL-33 release and innate Th2-type responses. *J Immunol.* 2011;186(7):4375–4387. [PubMed: 21357533]
21. Hristova M, Habibovic A, Veith C, et al. Airway epithelial dual oxidase 1 mediates allergen-induced IL-33 secretion and activation of type 2 immune responses. *J Allergy Clin Immunol.* 2015.
22. Bartemes KR, Iijima K, Kobayashi T, Kephart GM, McKenzie AN, Kita H. IL-33-responsive lineage- CD25+ CD44(hi) lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs. *J Immunol.* 2012;188(3):1503–1513. [PubMed: 22198948]
23. Barlow JL, Peel S, Fox J, et al. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J Allergy Clin Immunol.* 2013;132(4):933–941. [PubMed: 23810766]
24. Komai-Koma M, Xu D, Li Y, McKenzie AN, McInnes IB, Liew FY. IL-33 is a chemoattractant for human Th2 cells. *Eur J Immunol.* 2007;37(10):2779–2786. [PubMed: 17853410]
25. Traister RS, Uvalle CE, Hawkins GA, Meyers DA, Bleecker ER, Wenzel SE. Phenotypic and genotypic association of epithelial IL1RL1 to human TH2-like asthma. *J Allergy Clin Immunol.* 2015;135(1):92–99. [PubMed: 25091434]
26. Borish L The immunology of asthma: Asthma phenotypes and their implications for personalized treatment. *Ann Allergy Asthma Immunol.* 2016;117(2):108–114. [PubMed: 27499537]
27. Zhou B, Comeau MR, De ST, et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol.* 2005;6(10):1047–1053. [PubMed: 16142237]

28. Rochman Y, Dienger-Stambaugh K, Richgels PK, et al. TSLP signaling in CD4(+) T cells programs a pathogenic T helper 2 cell state. *Sci Signal*. 2018;11(521).
29. Kabata H, Moro K, Fukunaga K, et al. Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells during airway inflammation. *Nat Commun*. 2013;4:2675. [PubMed: 24157859]
30. Liu S, Verma M, Michalec L, et al. Steroid resistance of airway type 2 innate lymphoid cells from patients with severe asthma: The role of thymic stromal lymphopoietin. *J Allergy Clin Immunol*. 2018;141(1):257–268. [PubMed: 28433687]
31. Gauvreau GM, O'Byrne PM, Boulet LP, et al. Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. *N Engl J Med*. 2014;370(22):2102–2110. [PubMed: 24846652]
32. Corren J, Parnes JR, Wang L, et al. Tezepelumab in Adults with Uncontrolled Asthma. *N Engl J Med*. 2017;377(10):936–946. [PubMed: 28877011]
33. Andreaskos E, Papadopoulos NG. IL-25: the missing link between allergy, viral infection, and asthma? *Science translational medicine*. 2014;6(256):256fs238.
34. Corrigan CJ, Wang W, Meng Q, et al. T-helper cell type 2 (Th2) memory T cell-potentiating cytokine IL-25 has the potential to promote angiogenesis in asthma. *Proc Natl Acad Sci U S A*. 2011;108(4):1579–1584. [PubMed: 21205894]
35. Kouzaki H, Tojima I, Kita H, Shimizu T. Transcription of interleukin-25 and extracellular release of the protein is regulated by allergen proteases in airway epithelial cells. *Am J Respir Cell Mol Biol*. 2013;49(5):741–750. [PubMed: 23590308]
36. Rickel EA, Siegel LA, Yoon BR, et al. Identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25-induced activities. *J Immunol*. 2008;181(6):4299–4310. [PubMed: 18768888]
37. Su J, Chen T, Ji XY, et al. IL-25 downregulates Th1/Th17 immune response in an IL-10-dependent manner in inflammatory bowel disease. *Inflamm Bowel Dis*. 2013;19(4):720–728. [PubMed: 23429464]
38. Chang SH, Dong C. Signaling of interleukin-17 family cytokines in immunity and inflammation. *Cell Signal*. 2011;23(7):1069–1075. [PubMed: 21130872]
39. Ballantyne SJ, Barlow JL, Jolin HE, et al. Blocking IL-25 prevents airway hyperresponsiveness in allergic asthma. *J Allergy Clin Immunol*. 2007;120(6):1324–1331. [PubMed: 17889290]
40. Gregory LG, Jones CP, Walker SA, et al. IL-25 drives remodelling in allergic airways disease induced by house dust mite. *Thorax*. 2013;68(1):82–90. [PubMed: 23093652]
41. Cheng D, Xue Z, Yi L, et al. Epithelial interleukin-25 is a key mediator in Th2-high, corticosteroidresponsive asthma. *Am J Respir Crit Care Med*. 2014;190(6):639–648. [PubMed: 25133876]
42. Tang W, Smith SG, Beaudin S, et al. IL-25 and IL-25 receptor expression on eosinophils from subjects with allergic asthma. *Int Arch Allergy Immunol*. 2014;163(1):5–10. [PubMed: 24247484]
43. Willis CR, Siegel L, Leith A, et al. IL-17RA Signaling in Airway Inflammation and Bronchial Hyperreactivity in Allergic Asthma. *Am J Respir Cell Mol Biol*. 2015;53(6):810–821. [PubMed: 25919006]
44. Busse WW, Holgate S, Kerwin E, et al. Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. *Am J Respir Crit Care Med*. 2013;188(11):1294–1302. [PubMed: 24200404]
45. Loughnan MS, Takatsu K, Harada N, Nossal GJ. T-cell-replacing factor (interleukin 5) induces expression of interleukin 2 receptors on murine splenic B cells. *Proc Natl Acad Sci U S A*. 1987;84(15):5399–5403. [PubMed: 3110787]
46. Sanderson CJ, O'Garra A, Warren DJ, Klaus GG. Eosinophil differentiation factor also has B-cell growth factor activity: proposed name interleukin 4. *Proc Natl Acad Sci U S A*. 1986;83(2):437–440. [PubMed: 3484551]
47. Sanderson CJ, Warren DJ, Strath M. Identification of a lymphokine that stimulates eosinophil differentiation in vitro. Its relationship to interleukin 3, and functional properties of eosinophils produced in cultures. *J Exp Med*. 1985;162(1):60–74. [PubMed: 3925072]

48. Azuma C, Tanabe T, Konishi M, et al. Cloning of cDNA for human T-cell replacing factor (interleukin-5) and comparison with the murine homologue. *Nucleic Acids Res.* 1986;14(22): 9149–9158. [PubMed: 3024129]
49. Takatsu K Interleukin-5 and IL-5 receptor in health and diseases. *Proc Jpn Acad Ser B Phys Biol Sci.* 2011;87(8):463–485.
50. Tominaga A, Takaki S, Koyama N, et al. Transgenic mice expressing a B cell growth and differentiation factor gene (interleukin 5) develop eosinophilia and autoantibody production. *J Exp Med.* 1991;173(2):429–437. [PubMed: 1988543]
51. Iwama T, Nagai H, Suda H, Tsuruoka N, Koda A. Effect of murine recombinant interleukin-5 on the cell population in guinea-pig airways. *Br J Pharmacol.* 1992;105(1):19–22. [PubMed: 1596681]
52. Chand N, Harrison JE, Rooney S, et al. Anti-IL-5 monoclonal antibody inhibits allergic late phase bronchial eosinophilia in guinea pigs: a therapeutic approach. *Eur J Pharmacol.* 1992;211(1):121–123. [PubMed: 1618260]
53. Van Oosterhout AJ, Ladenius AR, Savelkoul HF, Van Ark I, Delsman KC, Nijkamp FP. Effect of anti-IL-5 and IL-5 on airway hyperreactivity and eosinophils in guinea pigs. *Am Rev Respir Dis.* 1993;147(3):548–552. [PubMed: 8442585]
54. Nakajima H, Iwamoto I, Tomoe S, et al. CD4+ T-lymphocytes and interleukin-5 mediate antigen-induced eosinophil infiltration into the mouse trachea. *Am Rev Respir Dis.* 1992;146(2): 374–377. [PubMed: 1362635]
55. Tanaka H, Kawada N, Yamada T, Kawada K, Takatsu K, Nagai H. Allergen-induced airway inflammation and bronchial responsiveness in interleukin-5 receptor alpha chain-deficient mice. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology.* 2000;30(6):874–881. [PubMed: 10848907]
56. Lee JJ, McGarry MP, Farmer SC, et al. Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. *J Exp Med.* 1997;185(12): 2143–2156. [PubMed: 9182686]
57. Lee JJ, Dimina D, Macias MP, et al. Defining a link with asthma in mice congenitally deficient in eosinophils. *Science.* 2004;305(5691):1773–1776. [PubMed: 15375267]
58. Jacobsen EA, Ochkur SI, Pero RS, et al. Allergic pulmonary inflammation in mice is dependent on eosinophil-induced recruitment of effector T cells. *J Exp Med.* 2008;205(3):699–710. [PubMed: 18316417]
59. Corrigan CJ, Haczku A, Gemou-Engesaeth V, et al. CD4 T-lymphocyte activation in asthma is accompanied by increased serum concentrations of interleukin-5. Effect of glucocorticoid therapy. *Am Rev Respir Dis.* 1993;147(3):540–547. [PubMed: 8095124]
60. Humbert M, Corrigan CJ, Kimmitt P, Till SJ, Kay AB, Durham SR. Relationship between IL-4 and IL-5 mRNA expression and disease severity in atopic asthma. *Am J Respir Crit Care Med.* 1997;156(3 Pt 1):704–708. [PubMed: 9309982]
61. van der Veen MJ, Van Neerven RJ, De Jong EC, Aalberse RC, Jansen HM, van der Zee JS. The late asthmatic response is associated with baseline allergen-specific proliferative responsiveness of peripheral T lymphocytes in vitro and serum interleukin-5. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology.* 1999;29(2):217–227.
62. Shi HZ, Xiao CQ, Zhong D, et al. Effect of inhaled interleukin-5 on airway hyperreactivity and eosinophilia in asthmatics. *Am J Respir Crit Care Med.* 1998;157(1):204–209. [PubMed: 9445301]
63. Leckie MJ, Jenkins GR, Khan J, et al. Sputum T lymphocytes in asthma, COPD and healthy subjects have the phenotype of activated intraepithelial T cells (CD69+ CD103+). *Thorax.* 2003;58(1):23–29. [PubMed: 12511714]
64. Minty A, Chalon P, Derocq JM, et al. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. *Nature.* 1993;362(6417):248–250. [PubMed: 8096327]
65. Ho IC, Vorhees P, Marin N, et al. Human GATA-3: a lineage-restricted transcription factor that regulates the expression of the T cell receptor alpha gene. *EMBO J.* 1991;10(5):1187–1192. [PubMed: 1827068]

66. Palmer-Crocker RL, Hughes CC, Pober JS. IL-4 and IL-13 activate the JAK2 tyrosine kinase and Stat6 in cultured human vascular endothelial cells through a common pathway that does not involve the gamma c chain. *J Clin Invest.* 1996;98(3):604–609. [PubMed: 8698849]
67. Kaur D, Hollins F, Woodman L, et al. Mast cells express IL-13R alpha 1: IL-13 promotes human lung mast cell proliferation and Fc epsilon RI expression. *Allergy.* 2006;61(9):1047–1053. [PubMed: 16918506]
68. Laporte JC, Moore PE, Baraldo S, et al. Direct effects of interleukin-13 on signaling pathways for physiological responses in cultured human airway smooth muscle cells. *Am J Respir Crit Care Med.* 2001;164(1):141–148. [PubMed: 11435252]
69. Lordan JL, Bucchieri F, Richter A, et al. Cooperative effects of Th2 cytokines and allergen on normal and asthmatic bronchial epithelial cells. *J Immunol.* 2002;169(1):407–414. [PubMed: 12077271]
70. Murata T, Husain SR, Mohri H, Puri RK. Two different IL-13 receptor chains are expressed in normal human skin fibroblasts, and IL-4 and IL-13 mediate signal transduction through a common pathway. *Int Immunol.* 1998;10(8):1103–1110. [PubMed: 9723696]
71. Wang IM, Lin H, Goldman SJ, Kobayashi M. STAT-1 is activated by IL-4 and IL-13 in multiple cell types. *Mol Immunol.* 2004;41(9):873–884. [PubMed: 15261459]
72. Newcomb DC, Boswell MG, Huckabee MM, et al. IL-13 regulates Th17 secretion of IL-17A in an IL-10-dependent manner. *J Immunol.* 2012;188(3):1027–1035. [PubMed: 22210911]
73. Newcomb DC, Boswell MG, Zhou W, et al. Human Th17 cells express a functional IL-13 receptor and IL-13 attenuates IL-17A production. *J Allergy Clin Immunol.* 2011;
74. Wilson MS, Ramalingam TR, Rivollier A, et al. Colitis and intestinal inflammation in IL10–/– mice results from IL-13Ralpha2-mediated attenuation of IL-13 activity. *Gastroenterology.* 2011;140(1):254–264. [PubMed: 20951137]
75. Hall SL, Baker T, Lajoie S, et al. IL-17A enhances IL-13 activity by enhancing IL-13-induced signal transducer and activator of transcription 6 activation. *J Allergy Clin Immunol.* 2017;139(2):462–471 e414. [PubMed: 27417023]
76. Grunig G, Warnock M, Wakil AE, et al. Requirement for IL-13 independently of IL-4 in experimental asthma [see comments]. *Science.* 1998;282(5397):2261–2263. [PubMed: 9856950]
77. Wills-Karp M, Luyimbazi J, Xu X, et al. Interleukin-13: central mediator of allergic asthma. *Science.* 1998;282(5397):2258–2261. [PubMed: 9856949]
78. Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP. IL-13 selectively induces vascular cell adhesion molecule-1 expression in human endothelial cells. *J Immunol.* 1995;154(2):799–803. [PubMed: 7529288]
79. Defrance T, Carayon P, Billian G, et al. Interleukin 13 is a B cell stimulating factor. *J Exp Med.* 1994;179(1):135–143. [PubMed: 7903680]
80. Punnonen J, Aversa G, Cocks BG, et al. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. *Proc Natl Acad Sci U S A.* 1993;90(8):3730–3734. [PubMed: 8097323]
81. Huang SK, Xiao HQ, Kleine-Tebbe J, et al. IL-13 expression at the sites of allergen challenge in patients with asthma. *J Immunol.* 1995;155(5):2688–2694. [PubMed: 7650396]
82. Kroegel C, Julius P, Matthys H, Virchow JC, Jr., Luttmann W. Endobronchial secretion of interleukin-13 following local allergen challenge in atopic asthma: relationship to interleukin-4 and eosinophil counts. *Eur Respir J.* 1996;9(5):899–904. [PubMed: 8793449]
83. Humbert M, Durham SR, Kimmitt P, et al. Elevated expression of messenger ribonucleic acid encoding IL-13 in the bronchial mucosa of atopic and nonatopic subjects with asthma. *J Allergy Clin Immunol.* 1997;99(5):657–665. [PubMed: 9155833]
84. Spahn JD, Szefer SJ, Surs W, Doherty DE, Nimmagadda SR, Leung DY. A novel action of IL-13: induction of diminished monocyte glucocorticoid receptor-binding affinity. *J Immunol.* 1996;157(6):2654–2659. [PubMed: 8805670]
85. Woodruff PG, Modrek B, Choy DF, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med.* 2009;180(5):388–395. [PubMed: 19483109]

86. Corren J, Lemanske RF, Hanania NA, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med*. 2011;365(12):1088–1098. [PubMed: 21812663]
87. Hanania NA, Noonan M, Corren J, et al. Lebrikizumab in moderate-to-severe asthma: pooled data from two randomised placebo-controlled studies. *Thorax*. 2015;70(8):748–756. [PubMed: 26001563]
88. Hanania NA, Korenblat P, Chapman KR, et al. Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. *Lancet Respir Med*. 2016;4(10):781–796. [PubMed: 27616196]
89. Korenblat P, Kerwin E, Leshchenko I, et al. Efficacy and safety of lebrikizumab in adult patients with mild-to-moderate asthma not receiving inhaled corticosteroids. *Respir Med*. 2018;134:143–149. [PubMed: 29413502]
90. Borish LC, Nelson HS, Lanz MJ, et al. Interleukin-4 receptor in moderate atopic asthma. A phase I/II randomized, placebo-controlled trial. *Am J Respir Crit Care Med*. 1999;160(6):1816–1823. [PubMed: 10588591]
91. Borish LC, Nelson HS, Corren J, et al. Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. *J Allergy Clin Immunol*. 2001;107(6):963–970. [PubMed: 11398072]
92. Wenzel S, Wilbraham D, Fuller R, Getz EB, Longphre M. Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. *Lancet*. 2007;370(9596):1422–1431. [PubMed: 17950857]
93. Corren J, Busse W, Meltzer EO, et al. A randomized, controlled, phase 2 study of AMG 317, an IL-4R α antagonist, in patients with asthma. *Am J Respir Crit Care Med*. 2010;181(8):788–796. [PubMed: 20056900]
94. Wenzel S, Ford L, Pearlman D, et al. Dupilumab in persistent asthma with elevated eosinophil levels. *N Engl J Med*. 2013;368(26):2455–2466. [PubMed: 23688323]
95. Wenzel S, Castro M, Corren J, et al. Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting β_2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. *Lancet*. 2016;388(10039):31–44. [PubMed: 27130691]
96. Krug N, Hohlfeld JM, Kirsten AM, et al. Allergen-induced asthmatic responses modified by a GATA3-specific DNase. *N Engl J Med*. 2015;372(21):1987–1995. [PubMed: 25981191]
97. Braddock M, Hanania NA, Sharafkhan A, Colice G, Carlsson M. Potential Risks Related to Modulating Interleukin-13 and Interleukin-4 Signalling: A Systematic Review. *Drug Saf*. 2018.
98. Paul WE. History of interleukin-4. *Cytokine*. 2015;75(1):3–7. [PubMed: 25814340]
99. Coffman RL, Ohara J, Bond MW, Carty J, Zlotnik A, Paul WE. B cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide-activated B cells. *J Immunol*. 1986;136(12):4538–4541. [PubMed: 3486902]
100. Sutherland GR, Baker E, Callen DF, et al. Interleukin 4 is at 5q31 and interleukin 6 is at 7p15. *Hum Genet*. 1988;79(4):335–337. [PubMed: 3261707]
101. Russell SM, Keegan AD, Harada N, et al. Interleukin-2 receptor gamma chain: a functional component of the interleukin-4 receptor. *Science*. 1993;262(5141):1880–1883. [PubMed: 8266078]
102. Swain SL, Weinberg AD, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. *J Immunol*. 1990;145(11):3796–3806. [PubMed: 2147202]
103. Zhu J, Yamane H, Cote-Sierra J, Guo L, Paul WE. GATA-3 promotes Th2 responses through three different mechanisms: induction of Th2 cytokine production, selective growth of Th2 cells and inhibition of Th1 cell-specific factors. *Cell Res*. 2006;16(1):3–10. [PubMed: 16467870]
104. Brown MA, Pierce JH, Watson CJ, Falco J, Ihle JN, Paul WE. B cell stimulatory factor-1/interleukin-4 mRNA is expressed by normal and transformed mast cells. *Cell*. 1987;50(5):809–818. [PubMed: 3497723]
105. Moqbel R, Ying S, Barkans J, et al. Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product. *J Immunol*. 1995;155(10):4939–4947. [PubMed: 7594499]

106. Nonaka M, Nonaka R, Woolley K, et al. Distinct immunohistochemical localization of IL-4 in human inflamed airway tissues. IL-4 is localized to eosinophils in vivo and is released by peripheral blood eosinophils. *J Immunol*. 1995;155(6):3234–3244. [PubMed: 7673736]
107. Plaut M, Pierce JH, Watson CJ, Hanley-Hyde J, Nordan RP, Paul WE. Mast cell lines produce lymphokines in response to cross-linkage of Fc epsilon RI or to calcium ionophores. *Nature*. 1989;339(6219):64–67. [PubMed: 2469965]
108. Seder RA, Paul WE, Dvorak AM, et al. Mouse splenic and bone marrow cell populations that express high-affinity Fc epsilon receptors and produce interleukin 4 are highly enriched in basophils. *Proc Natl Acad Sci U S A*. 1991;88(7):2835–2839. [PubMed: 1826367]
109. Yoshimoto T, Bendelac A, Watson C, Hu-Li J, Paul WE. Role of NK1.1+ T cells in a TH2 response and in immunoglobulin E production. *Science*. 1995;270(5243):1845–1847. [PubMed: 8525383]
110. Doherty TA, Khorram N, Lund S, Mehta AK, Croft M, Broide DH. Lung type 2 innate lymphoid cells express cysteinyl leukotriene receptor 1, which regulates TH2 cytokine production. *J Allergy Clin Immunol*. 2013;132(1):205–213. [PubMed: 23688412]
111. Bochner BS, Luscinskas FW, Gimbrone MA, Jr., et al. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. *J Exp Med*. 1991;173(6):1553–1557. [PubMed: 1709678]
112. Masinovskiy B, Urdal D, Gallatin WM. IL-4 acts synergistically with IL-1 beta to promote lymphocyte adhesion to microvascular endothelium by induction of vascular cell adhesion molecule-1. *J Immunol*. 1990;145(9):2886–2895. [PubMed: 1698865]
113. Temann UA, Prasad B, Gallup MW, et al. A novel role for murine IL-4 in vivo: induction of MUC5AC gene expression and mucin hypersecretion. *Am J Respir Cell Mol Biol*. 1997;16(4):471–478. [PubMed: 9115759]
114. Brusselle GG, Kips JC, Tavernier JH, et al. Attenuation of allergic airway inflammation in IL-4 deficient mice [see comments]. *Clinical & Experimental Allergy*. 1994;24(1):73–80. [PubMed: 8156448]
115. Coyle AJ, Le Gros G, Bertrand C, et al. Interleukin-4 is required for the induction of lung Th2 mucosal immunity. *American Journal of Respiratory Cell & Molecular Biology*. 1995;13(1):54–59. [PubMed: 7598937]
116. Corry DB, Folkesson HG, Warnock ML, et al. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity [see comments]. *J Exp Med*. 1996;183(1):109–117. [PubMed: 8551213]
117. Robinson DS, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med*. 1992;326(5):298–304. [PubMed: 1530827]
118. Walker C, Bode E, Boer L, Hansel TT, Blaser K, Virchow JC, Jr. Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. *Am Rev Respir Dis*. 1992;146(1):109–115. [PubMed: 1626792]
119. Robinson D, Hamid Q, Ying S, et al. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell interleukin-4, interleukin-5, and interferon-gamma cytokine gene expression. *Am Rev Respir Dis*. 1993;148(2):401–406. [PubMed: 8342904]
120. Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol*. 1993;92(2):313–324. [PubMed: 8349942]
121. Holgate ST. Innate and adaptive immune responses in asthma. *Nat Med*. 2012;18(5):673–683. [PubMed: 22561831]
122. Interleukin Veldhoen M. 17 is a chief orchestrator of immunity. *Nat Immunol*. 2017;18(6):612–621. [PubMed: 28518156]
123. Newcomb DC, Peebles RS, Jr. Th17-mediated inflammation in asthma. *Curr Opin Immunol*. 2013;25(6):755–760. [PubMed: 24035139]
124. Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. *Nat Rev Immunol*. 2010;10(7):479–489. [PubMed: 20559326]

125. Miossec P, Kolls JK. Targeting IL-17 and TH17 cells in chronic inflammation. *Nat Rev Drug Discov.* 2012;11(10):763–776. [PubMed: 23023676]
126. Tamassia N, Arruda-Silva F, Calzetti F, et al. A Reappraisal on the Potential Ability of Human Neutrophils to Express and Produce IL-17 Family Members In Vitro: Failure to Reproducibly Detect It. *Front Immunol.* 2018;9:795. [PubMed: 29719541]
127. Toy D, Kugler D, Wolfson M, et al. Cutting edge: interleukin 17 signals through a heteromeric receptor complex. *J Immunol.* 2006;177(1):36–39. [PubMed: 16785495]
128. Ghoreschi K, Laurence A, Yang XP, et al. Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. *Nature.* 2010;467(7318):967–971. [PubMed: 20962846]
129. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol.* 2009;27:485–517. [PubMed: 19132915]
130. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity.* 2006;24(2):179–189. [PubMed: 16473830]
131. Schnyder-Candrian S, Togbe D, Couillin I, et al. Interleukin-17 is a negative regulator of established allergic asthma. *J Exp Med.* 2006;203(12):2715–2725. [PubMed: 17101734]
132. Lajoie S, Lewkowich IP, Suzuki Y, et al. Complement-mediated regulation of the IL-17A axis is a central genetic determinant of the severity of experimental allergic asthma. *Nat Immunol.* 2010;11(10):928–935. [PubMed: 20802484]
133. McKinley L, Alcorn JF, Peterson A, et al. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J Immunol.* 2008;181(6):4089–4097. [PubMed: 18768865]
134. Barlow JL, Flynn RJ, Ballantyne SJ, McKenzie AN. Reciprocal expression of IL-25 and IL-17A is important for allergic airways hyperreactivity. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology.* 2011;41(10):1447–1455.
135. Molet S, Hamid Q, Davoine F, et al. IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J Allergy Clin Immunol.* 2001;108(3):430–438. [PubMed: 11544464]
136. Doe C, Bafadhel M, Siddiqui S, et al. Expression of the T helper 17-associated cytokines IL-17A and IL-17F in asthma and COPD. *Chest.* 2010;138(5):1140–1147. [PubMed: 20538817]
137. Bullens DM, Truyen E, Coteur L, et al. IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? *Respir Res.* 2006;7:135. [PubMed: 17083726]
138. Saleh A, Shan L, Halayko AJ, Kung S, Gounni AS. Critical role for STAT3 in IL-17A-mediated CCL11 expression in human airway smooth muscle cells. *J Immunol.* 2009;182(6):3357–3365. [PubMed: 19265112]
139. Inoue D, Numasaki M, Watanabe M, et al. IL-17A promotes the growth of airway epithelial cells through ERK-dependent signaling pathway. *Biochem Biophys Res Commun.* 2006;347(4):852–858. [PubMed: 16859642]
140. Chen Y, Thai P, Zhao YH, Ho YS, DeSouza MM, Wu R. Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem.* 2003;278(19):17036–17043. [PubMed: 12624114]
141. Fujisawa T, Velichko S, Thai P, Hung LY, Huang F, Wu R. Regulation of airway MUC5AC expression by IL-1beta and IL-17A; the NF-kappaB paradigm. *J Immunol.* 2009;183(10):6236–6243. [PubMed: 19841186]
142. Chang Y, Al-Alwan L, Risse PA, et al. Th17-associated cytokines promote human airway smooth muscle cell proliferation. *FASEB J.* 2012;26(12):5152–5160. [PubMed: 22898922]
143. Chang Y, Al-Alwan L, Risse PA, et al. TH17 cytokines induce human airway smooth muscle cell migration. *J Allergy Clin Immunol.* 2011;127(4):1046–1053 [PubMed: 21345484]
144. Rusta-Sallehy S, Gooderham M, Papp K. Brodalumab: A Review of Safety. *Skin Therapy Lett.* 2018;23(2):1–3.
145. Harizi H, Juzan M, Moreau JF, Gualde N. Prostaglandins inhibit 5-lipoxygenase-activating protein expression and leukotriene B4 production from dendritic cells via an IL-10-dependent mechanism. *J Immunol.* 2003;170(1):139–146. [PubMed: 12496393]

146. Peebles RS, Jr., Boyce JA. Lipid mediators of hypersensitivity and inflammation In: Adkinson NF, Jr., Bochner BS, Burks AW, et al., eds. Middleton's Allergy Principles and Practice. Vol 1 Eighth ed. Philadelphia, PA: Elsevier; 2014:139–161.
147. Smith WL, Urade Y, Jakobsson PJ. Enzymes of the cyclooxygenase pathways of prostanoid biosynthesis. *Chem Rev.* 2011;111(10):5821–5865. [PubMed: 21942677]
148. Luna-Gomes T, Magalhaes KG, Mesquita-Santos FP, et al. Eosinophils as a novel cell source of prostaglandin D2: autocrine role in allergic inflammation. *J Immunol.* 2011;187(12):6518–6526. [PubMed: 22102725]
149. Mutalithas K, Guillen C, Day C, Brightling CE, Pavord ID, Wardlaw AJ. CRTH2 expression on T cells in asthma. *Clin Exp Immunol.* 2010;161(1):34–40. [PubMed: 20491797]
150. Barnig C, Cernadas M, Dutile S, et al. Lipoxin A4 regulates natural killer cell and type 2 innate lymphoid cell activation in asthma. *Sci Transl Med.* 2013;5(174):174ra126.
151. Xue L, Salimi M, Panse I, et al. Prostaglandin D2 activates group 2 innate lymphoid cells through chemoattractant receptor-homologous molecule expressed on TH2 cells. *J Allergy Clin Immunol.* 2014;133(4):1184–1194. [PubMed: 24388011]
152. Salimi M, Stoger L, Liu W, et al. Cysteinyl leukotriene E4 activates human group 2 innate lymphoid cells and enhances the effect of prostaglandin D2 and epithelial cytokines. *J Allergy Clin Immunol.* 2017;140(4):1090–1100.e1011. [PubMed: 28115217]
153. Murray M, Webb MS, O'Callaghan C, Swarbrick AS, Milner AD. Respiratory status and allergy after bronchiolitis. *Archives of Disease in Childhood.* 1992;67(4):482–487. [PubMed: 1580676]
154. Fajt ML, Gelhaus SL, Freeman B, et al. Prostaglandin D(2) pathway upregulation: relation to asthma severity, control, and TH2 inflammation. *J Allergy Clin Immunol.* 2013;131(6):1504–1512. [PubMed: 23506843]
155. Naclerio RM, Meier HL, Kagey-Sobotka A, et al. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis.* 1983;128(4):597–602. [PubMed: 6354022]
156. Proud D, Sweet J, Stein P, et al. Inflammatory mediator release on conjunctival provocation of allergic subjects with allergen. *J Allergy Clin Immunol.* 1990;85(5):896–905. [PubMed: 1692049]
157. Charlesworth EN, Kagey-Sobotka A, Schleimer RP, Norman PS, Lichtenstein LM. Prednisone inhibits the appearance of inflammatory mediators and the influx of eosinophils and basophils associated with the cutaneous late- phase response to allergen. *J Immunol.* 1991;146(2):671676.
158. Barnes N, Pavord I, Chuchalin A, et al. A randomized, double-blind, placebo-controlled study of the CRTH2 antagonist OC000459 in moderate persistent asthma. *Clin Exp Allergy.* 2012;42(1):38–48. [PubMed: 21762224]
159. Straumann A, Hoesli S, Bussmann C, et al. Anti-eosinophil activity and clinical efficacy of the CRTH2 antagonist OC000459 in eosinophilic esophagitis. *Allergy.* 2013;68(3):375–385. [PubMed: 23379537]
160. Krug N, Gupta A, Badorrek P, et al. Efficacy of the oral chemoattractant receptor homologous molecule on TH2 cells antagonist BI 671800 in patients with seasonal allergic rhinitis. *J Allergy Clin Immunol.* 2014;133(2):414–419. [PubMed: 24332218]
161. Hall IP, Fowler AV, Gupta A, et al. Efficacy of BI 671800, an oral CRTH2 antagonist, in poorly controlled asthma as sole controller and in the presence of inhaled corticosteroid treatment. *Pulmonary pharmacology & therapeutics.* 2015;32:37–44. [PubMed: 25861737]
162. Miller D, Wood C, Bateman E, et al. A randomized study of BI 671800, a CRTH2 antagonist, as add-on therapy in poorly controlled asthma. *Allergy Asthma Proc.* 2017;38(2):157–164. [PubMed: 28234053]
163. Erpenbeck VJ, Popov TA, Miller D, et al. The oral CRTh2 antagonist QAW039 (feviprant): A phase II study in uncontrolled allergic asthma. *Pulmonary pharmacology & therapeutics.* 2016;39:54–63. [PubMed: 27354118]
164. Gonem S, Berair R, Singapuri A, et al. Feviprant, a prostaglandin D2 receptor 2 antagonist, in patients with persistent eosinophilic asthma: a single-centre, randomised, double-blind, parallelgroup, placebo-controlled trial. *Lancet Respir Med.* 2016;4(9):699–707. [PubMed: 27503237]

165. Kuna P, Bjermer L, Tornling G. Two Phase II randomized trials on the CRTh2 antagonist AZD1981 in adults with asthma. Drug design, development and therapy. 2016;10:2759–2770.
166. Hsieh FH, Lam BK, Penrose JF, Austen KF, Boyce JA. T helper cell type 2 cytokines coordinately regulate immunoglobulin E-dependent cysteinyl leukotriene production by human cord blood-derived mast cells: profound induction of leukotriene C(4) synthase expression by interleukin 4. J Exp Med. 2001;193(1):123–133. [PubMed: 11136826]
167. Figueroa DJ, Breyer RM, Defoe SK, et al. Expression of the cysteinyl leukotriene 1 receptor in normal human lung and peripheral blood leukocytes. Am J Respir Crit Care Med. 2001;163(1):226–233. [PubMed: 11208650]
168. Lynch KR, O'Neill GP, Liu Q, et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. Nature. 1999;399(6738):789–793. [PubMed: 10391245]
169. Mellor EA, Frank N, Soler D, et al. Expression of the type 2 receptor for cysteinyl leukotrienes (CysLT2R) by human mast cells: Functional distinction from CysLT1R. Proc Natl Acad Sci U S A. 2003;100(20):11589–11593. [PubMed: 13679572]
170. Matsuda M, Tabuchi Y, Nishimura K, et al. Increased expression of CysLT2 receptors in the lung of asthmatic mice and role in allergic responses. Prostaglandins Leukot Essent Fatty Acids. 2018;131:24–31. [PubMed: 29628047]
171. Kanaoka Y, Maekawa A, Austen KF. Identification of GPR99 protein as a potential third cysteinyl leukotriene receptor with a preference for leukotriene E4 ligand. J Biol Chem. 2013;288(16):10967–10972. [PubMed: 23504326]
172. Bankova LG, Lai J, Yoshimoto E, et al. Leukotriene E4 elicits respiratory epithelial cell mucin release through the G-protein-coupled receptor, GPR99. Proc Natl Acad Sci U S A. 2016;113(22):6242–6247. [PubMed: 27185938]
173. Dube LM, Swanson LJ, Awni W. Zileuton, a leukotriene synthesis inhibitor in the management of chronic asthma. Clinical pharmacokinetics and safety. Clin Rev Allergy Immunol. 1999;17(1–2):213–221. [PubMed: 10436867]
174. Findlay SR, Barden JM, Easley CB, Glass M. Effect of the oral leukotriene antagonist, ICI 204,219, on antigen-induced bronchoconstriction in subjects with asthma. J Allergy Clin Immunol. 1992;89(5):1040–1045. [PubMed: 1583246]
175. Taylor IK, O'Shaughnessy KM, Fuller RW, Dollery CT. Effect of cysteinyl-leukotriene receptor antagonist ICI 204.219 on allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects. Lancet. 1991;337(8743):690–694. [PubMed: 1672176]
176. Calhoun WJ, Lavins BJ, Minkwitz MC, Evans R, Gleich GJ, Cohn J. Effect of zafirlukast (Accolate) on cellular mediators of inflammation: bronchoalveolar lavage fluid findings after segmental antigen challenge. Am J Respir Crit Care Med. 1998;157(5 Pt 1):1381–1389. [PubMed: 9603112]
177. Kane GC, Pollice M, Kim CJ, et al. A controlled trial of the effect of the 5-lipoxygenase inhibitor, zileuton, on lung inflammation produced by segmental antigen challenge in human beings. J Allergy Clin Immunol. 1996;97(2):646–654. [PubMed: 8621850]
178. Finnerty JP, Wood-Baker R, Thomson H, Holgate ST. Role of leukotrienes in exercise-induced asthma. Inhibitory effect of ICI 204219, a potent leukotriene D4 receptor antagonist. Am Rev Respir Dis. 1992;145(4 Pt 1):746–749. [PubMed: 1554195]
179. Leff JA, Busse WW, Pearlman D, et al. Montelukast, a leukotriene-receptor antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. N Engl J Med. 1998;339(3):147–152. [PubMed: 9664090]
180. Meltzer SS, Hasday JD, Cohn J, Bleecker ER. Inhibition of exercise-induced bronchospasm by zileuton: a 5-lipoxygenase inhibitor. Am J Respir Crit Care Med. 1996;153(3):931–935. [PubMed: 8630575]
181. Reiss TF, Hill JB, Harman E, et al. Increased urinary excretion of LTE4 after exercise and attenuation of exercise-induced bronchospasm by montelukast, a cysteinyl leukotriene receptor antagonist. Thorax. 1997;52(12):1030–1035. [PubMed: 9516894]
182. Śladek K, Szczeklik A. Cysteinyl leukotrienes overproduction and mast cell activation in aspirin-provoked bronchospasm in asthma. Eur Respir J. 1993;6(3):391–399. [PubMed: 8386106]

183. Christie PE, Smith CM, Lee TH. The potent and selective sulfidopeptide leukotriene antagonist, SK&F 104353, inhibits aspirin-induced asthma. *Am Rev Respir Dis.* 1991;144(4):957–958. [PubMed: 1928974]
184. Fischer AR, Rosenberg MA, Lilly CM, et al. Direct evidence for a role of the mast cell in the nasal response to aspirin in aspirin-sensitive asthma. *J Allergy Clin Immunol.* 1994;94(6 Pt 1): 1046–1056. [PubMed: 7798537]
185. Israel E, Fischer AR, Rosenberg MA, et al. The pivotal role of 5-lipoxygenase products in the reaction of aspirin-sensitive asthmatics to aspirin. *Am Rev Respir Dis.* 1993;148(6 Pt 1):1447–1451. [PubMed: 8256883]
186. Kumlin M, Dahlen B, Bjorck T, Zetterstrom O, Granstrom E, Dahlen SE. Urinary excretion of leukotriene E4 and 11-dehydro-thromboxane B2 in response to bronchial provocations with allergen, aspirin, leukotriene D4, and histamine in asthmatics. *Am Rev Respir Dis.* 1992;146(1): 96–103. [PubMed: 1320824]

Key Points:

1. An important component of the innate allergic immune response is ILC2 activated by IL-33, TSLP, and IL-25 to produce IL-5 and IL-13
2. CD4 Th2 cells are an important component of the adaptive allergic immune response and produce IL-4, IL-5, and IL-13 in response to specific antigenic stimulation.
3. CD4 Th17 cells produce IL-17A and IL-17F that in turn induce the production of cytokines and chemokines that promote the chemotaxis and survival of neutrophils in the airway and lung.
4. Eicosanoids involved in asthma pathogenesis include PGD₂ and the cysteinyl leukotrienes that promote smooth muscle constriction and inflammation that propagate allergic responses.

Synopsis

There are multiple proinflammatory pathways in the pathogenesis of asthma. These include both innate and adaptive inflammation, in addition to inflammatory and physiologic responses mediated by eicosanoids. An important component of the innate allergic immune response is ILC2 activated by IL-33, TSLP, and IL-25 to produce IL-5 and IL-13. In terms of the adaptive T lymphocyte immunity, CD4 Th2 and IL-17-producing cells are critical in the inflammatory responses in asthma. Lastly, eicosanoids involved in asthma pathogenesis include PGD₂ and the cysteinyl leukotrienes that promote smooth muscle constriction and inflammation that propagate allergic responses.

Innate Allergic Airway Inflammation

Figure 1:
Pathway of innate allergic inflammation

Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens

Figure 2:
Pathway of adaptive allergic inflammation

IL-17A Mediated Airway Pathology

Figure 3:
Pathway of Th17 mediated inflammatory responses

Arachidonic Acid Metabolism

Figure 4:
Metabolism of Arachidonic Acid through the cyclooxygenase (COX) and 5-lipoxygenase (5-LO) pathways