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Key Mediators in the Immunopathogenesis of Allergic Asthma

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Abstract

Asthma is described as a chronic inflammatory disorder of the conducting airways. It is characterized by reversible airway obstruction, eosinophil and Th2 infiltration, airway hyper-responsiveness and airway remodeling. Our findings to date have largely been dependent on work done using animal models, which have been instrumental in broadening our understanding of the mechanism of the disease. However, using animals to model a uniquely human disease is not without its drawbacks. This review aims to examine some of the key mediators and cells of allergic asthma learned from animal models and shed some light on emerging mediators in the pathogenesis allergic airway inflammation in acute and chronic asthma.

Keywords

Airway epithelium; Airway hyper-responsiveness; Allergic airway inflammation; Asthma; Innate lymphoid cells; Surfactant Protein; Th17 cells

Introduction

Asthma is a complex disease involving multiple genetic and environmental influences (1). Linkage and association studies have identified various chromosome regions with hallmark features of asthma (2). There are approximately 300 million people worldwide who suffer from asthma and in next 10–15 years it is estimated to rise to more than 400 million (3).

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Characteristics of Allergic Asthma

Inflammation caused by human allergic asthma involves interplay of the respiratory epithelium, the adaptive and innate immune systems. This interaction drives a chronic response, which leads to remodeling of airways, with even the small airways becoming altered with increased chronicity (1, 4). The most prevalent form of asthma is atopic asthma where individuals have a genetic predisposition for the development of an antigen specific IgE mediated response to common aeroallergens (2, 5). Dendritic cells in the airway epithelium and submucosa detect inhaled allergens, including house dust mite (HDM), pollen, fungal spores and cockroach antigen. The IgE antibody bound to high affinity receptors on dendritic cells facilitates the uptake and internalization of these allergens (6, 7). Dendritic cells then migrate to the secondary lymphatic systems where they process and present antigens via MHC (major histocompatibility complex) class II to T- and B-lymphocytes. B-lymphocytes produce IgE, which binds to high affinity FcεRI on basophils and mast cells (5). Re-exposure to allergen causes crosslinking of receptors resulting in the release of mediators, including histamine, prostaglandins, leukotrienes, cytokines and chemokines (4, 5). These mediators are capable of contracting airway smooth muscle cells and inducing edema and mucous secretion. Chemokines attract a host of inflammatory cells, namely eosinophils, macrophages, neutrophils and T-lymphocytes (8). The products released from activated leukocytes lay the foundation for airway hyper-responsiveness and airway remodeling by causing damage to epithelial layers, promoting bronchoconstriction, and deposition of extracellular matrices (4, 8). Selective expansion of Th2 cells results in the secretion of cytokines, including interleukin 5 (IL-5) that causes airway eosinophilia, and IL-4 and IL-13 that induce goblet cell metaplasia and airway hyper-responsiveness (9). Persistent changes in airway structure can occur overtime resulting in goblet cell hyperplasia, sub-epithelial fibrosis and increased deposition of collagen throughout the airway smooth muscle (1).

Why do we need animal models?

Humans are the ideal subjects for studying disease pathogenesis. However, for ethical and logistic reasons, most *in vivo* experiments are carried out in experimental animals (1, 10). Animal models of asthma afford us the opportunity to design and conduct studies using intact immune and respiratory systems and give us a better understanding of their interaction in the lung (10, 11). The experimental animal models also allow testing the safety and efficacy of new drugs and therapeutic agents (1). Although there is no perfect model, several species (rodents, horses, sheep, non-human primates) and study designs (acute vs. chronic) have been employed to model allergic airway inflammation and asthma (12–14). Animal models of allergic airway inflammation can be credited for highlighting the importance of the Th2 phenotype and various cytokines and chemokines in the development and progression of asthma (10).

It is important to note that most animals used to study asthma do not spontaneously develop the disease (with the exception of cats and horses), therefore, they have to be sensitized and challenged with allergens to develop asthmatic-like immune reactions (12). Due to the complexity of asthma, some models are more suitable for studying the disease than others

depending on both practical and research considerations. It is, however, unlikely that a single animal model will be able to replicate all the morphological and clinical features of asthma (11).

Animal Models of Allergic Asthma

Small animals like mice, rats, and guinea pigs are widely used as animal models of allergic asthma and have proven to be valuable for the investigation of potential underlying mechanisms of airway pathophysiology both *in vivo* and *ex vivo*. They are often preferred models because they are relatively inexpensive, easy to handle with more species-specific reagents and equipment available for investigating airway pathology (15). The relative merit of each species is highlighted in Table 1.

Larger animals (dogs, sheep, horses and nonhuman primates) have been employed to study airway inflammation and remodeling based on natural or active sensitization to allergens and similarities in gross anatomy to that of a small human (11, 13). Although large animals are useful especially for results that are intended for translation to humans, they are hard to handle, generally expensive and less is known about the disease mechanisms in these models when compared to smaller animals (11, 14).

Mouse models of allergic asthma

Most asthma related research continues to be pursued using mouse models due to the vast number of immunological and molecular techniques that are available to study them. The development of transgenic and knockout mice has afforded asthma researchers with new technology that enables more comprehensive analysis of mediators involved with the disease (16).

Mice do not naturally develop asthma, so, an artificial allergic asthmatic-like reaction has to be induced in the airways. There are various sensitization and challenge protocols developed using different strains of mice (Table 2). The most commonly used strain for allergen challenge is the Balb/C mice because they are known to be high IgE responders to many allergens (17). Female mice are preferred because they develop greater eosinophilia, influx of immune cells and Th2 responses than their male counterparts (13).

The acute model of asthma usually lasts for about 6–8 weeks. Sensitization with a foreign allergen is often done in the presence of an adjuvant with follow up booster 1–2 weeks later (14). After an immune response has been developed the animal is challenged over several days and invasive and noninvasive techniques are used to assess pulmonary function (18).

Chronic models incorporate weeks to months of allergen challenge in sensitized mice done either by repeated exposure to low levels of antigens or over expression of cytokines in transgenic mice (19, 20). Some of these animal models have been successful in eliciting hallmark features of chronic asthma, including airway remodeling, epithelial cell hypertrophy and sub-epithelial fibrosis (20) and are valuable to examine relationship between mediators and cell types in the development of remodeling events (21).

Acute/Early Phase Response

Studies done using animal models have vastly improved our knowledge on the mediators and cells involved in the pathology of allergic asthma. Work done primarily with mice has highlighted that asthma involves interaction with the innate and adaptive immune system, which results in the production of cytokines, chemokines, cysteinyl leukotrienes and other emerging inflammatory mediators (19). Acute or early phase responses are characterized by bronchospasm mediated by normal resident cells in the epithelium such as mast cells, which release histamine, prostaglandin D₂, leukotriene D₄ and E₄. These mediators lead to changes in smooth muscle vasculature, which enables infiltration of cells, which release late phase mediators that contribute to chronic inflammation and airway remodeling (22).

Cells involved in Acute Inflammation

Mast Cells—Mast cells play a critical role in immediate allergic reactions and release potent mediators, including histamine, prostaglandins, chemokines, cytokines and leukotrienes that act on smooth muscle and inflammatory cells (23). They express FcεRI on their cell surface, which allows them to bind IgE unbound to antigen. Re-exposure to this antigen causes crosslinking of receptors which results in the activation and degranulation of mast cells to release preformed mediators like histamine and tryptase and synthesize newly generated mediators such as leukotriene (LT)C₄, prostaglandin (PG)D₂, platelet activating factor (PAF) and cytokines (23, 24).

Mice lacking FcεRIα chain had decreased bronchoalveolar lavage eosinophilia and IL-4 levels and diminished airway inflammation, suggesting the role of mast cells in the late phase response (25). Studies in human asthmatics have identified the presence of mast cells in lung submucosa, smooth muscle and airway epithelium, supporting the role of mast cells in the pathophysiology of asthma (26). Recently, it was shown that isolated human mast cells could regulate airway smooth muscle cell production of CXCL-10, which further attracts mast cells to the airways (27).

Basophils

Basophils have many features similar to that of mast cells. They are activated by multivalent antigen bound to IgE, which causes crosslinking of receptors and subsequent exocytosis of granules (28). The main preformed mediator released from basophils is histamine and they rapidly synthesize LTC₄, LTD₄ and LTE₄ upon activation (29). Basophils contribute to the propagation of allergic airway inflammation. They produce IL-4 that drives Th2 cell differentiation and proliferation (30). Studies in animal models suggest that basophils could stimulate a Th2 response in naïve CD4⁺ T-cells. In the same study, it was also observed that IL-4 deficient basophils failed to induce this response (31). Basophils have also been found to have the capacity to function as antigen presenting cells. Recent studies have emerged to suggest that basophils may possess the ability to travel to the draining lymph nodes and present antigen to T-cells further driving the Th2 polarization (30, 32). Clearly, the functions of basophils are not as redundant to mast cells as previously thought. With the generation of new transgenic animal models, more in-depth studies in the mechanism and actions of basophils can be facilitated in the future (33).

Innate Lymphoid Cells

Innate lymphoid cells (ILCs) are emerging mediators in the pathogenesis of asthma. They are involved in the promotion of rapid cytokine-dependent innate immunity, inflammation, remodeling and tissue repair (34). ILCs exhibit several features similar to CD4⁺ T-cells, such as development from a common lymphoid progenitor as well as transcription factors and cytokines which they secrete (35). There are broadly three groups of ILCs: ILC1, which express transcription factor T-bet and secrete IFN- γ and IL-12, (like Th1 cells); ILC2, which express transcription factor GATA-3 and produce IL-5, IL-9 and IL-13 (like Th2 cells); and ILC-3, which express ROR γ t and produce IL-17 and IL-22 (like Th17 cells) (35–37).

ILC2 were first described in mice and have been found to be involved in early phase responses of anti-helminthic and allergic responses (36). Though these cells do not express antigen-specific receptors they are capable of releasing Th2 cytokines upon activation by IL-33 and/or IL-25 (38). Barlow et al. (39) showed that ILC2 infiltrated the lungs after allergen challenge and were a major source of IL-13. Intranasal administration of IL-33 increased IL-13 producing ILCs and adoptive transfer of these cells to IL-13 null mice (usually resistant to developing IL-25-induced AHR) developed AHR and lung inflammation (39). ILC2 cells also express CysLT1R, which when binding to its ligand (LT_{D4}) induces production of IL-4, IL-5 and IL-13 from these cells contributing to the pathogenesis of asthma (40).

Much less is known about the involvement of ILC2s in the pathogenesis of human asthma as most of the studies done to date have been conducted in mice (41). There is, however, evidence to suggest that IL-25 and IL-33 promote recruitment and expansion of ILC2s and the production of IL-13 in human lungs (42). ILC2s have also been suggested to play a role in influencing adaptive responses but the mechanisms involved in this process is yet to be ascertained. It is obvious that further research into such novel cells is integral for better understanding of their mechanism of action and potential target for treatment.

Mediators of Acute Inflammation

IgE—Immunoglobulin E (IgE) has been associated with allergic reactions for some time and has been extensively researched and reviewed by many investigators. Of all the five classes of antibodies, IgE concentration is the lowest in the serum, which is, in part, due to the small number of B-cells differentiated into plasma cells and committed to its synthesis as well as its rapid absorption in tissues where it becomes tightly bound to its receptors (43). IgE is produced when differentiated B-cells undergo isotype switching. This is a recombination process which occurs at the molecular level and is mediated by interaction with T-lymphocytes (CD40:CD40L interaction) which have been previously activated by allergen presentation through antigen presenting cells (24, 44). Th2 cytokines (IL-4 and IL-13) secreted in the vicinity of the cells, turn on the ϵ germline transcript and activate STAT-6, which binds to the I ϵ promoter to initiate germline transcription, ultimately resulting in class switching to IgE (45). There are two IgE receptors, Fc ϵ RI (high affinity receptor primarily present on mast cells and basophils) and the Fc ϵ RII (low affinity receptor found on many cells including B-cells, T-cells, dendritic cells and other hematopoietic cells) (28).

The primary function of IgE is its role in immediate Type I hypersensitivity reactions. The presence of elevated levels of allergen specific serum IgE against otherwise harmless environmental antigens is strongly associated with atopic diseases. When a sensitized person comes in contact with an allergen, cross-linking of receptors cause degranulation of mast cells, which release potent mediators (46). IgE is also involved in the late phase responses. Research has shown that IgE stimulated mast cells produces a range of chemokines and cytokines that causes migration of immune cells associated with allergy (discussed further in next section). Studies using IgE-deficient mice as well as anti-IgE monoclonal antibody (omalizumab) have highlighted that IgE plays a role in the survival, proliferation, activation and migration of mast cells (47).

The relationship between asthma and IgE levels have been clearly elucidated and reviewed comprehensively with studies showing that IgE levels are higher in patients with atopic asthma than non-atopic individuals (48, 49). These elevated levels represent an important tool for allergy diagnosis. Local production of IgE may account for generally higher allergen-specific fractions in the tissue than in the blood. Although survival of IgE can be markedly increased by binding to its high affinity receptor, allergen specific IgE may stem wholly or partly from other sources (50). Research is ongoing to conclusively decipher the source that continuously replenishes serum IgE levels.

Histamine—Histamine is released from mast cells and basophils. Histamine can induce smooth muscle contraction, mucus production in goblet cells and nitric oxide production (51). Histamine has four receptors (H1R, H2R, H3R and H4R), which are found on a variety of cells involved in both the adaptive and innate immune response (52). H1Rs are found on smooth muscle cells, epithelial cells and leukocytes (53). The involvement of H1Rs in asthmatic reactions has been highlighted in studies where H1R knockout mice showed attenuated responses after allergen challenge and failed to develop airway inflammation (54). H4R, found on similar cell types as the H1R, have also been suggested to have a pro-inflammatory role in asthma. Results from studies using H4R knockout Balb/c mice treated with H4R antagonist showed decreased eosinophilia and Th2 responses (55). In another study, H4R antagonist was found to reduce airway hyperreactivity and inflammation with subsequent upregulation of transcription factor, forkhead box P3 (FOXP3), associated with CD4+CD25+ T regulatory cells, suggesting a possible target of suppression in asthmatic subjects (56).

Prostaglandin D₂—PGD₂ is the major cyclooxygenase metabolite of the arachidonic acid pathway produced by mast cells upon allergen challenge. PGD₂ causes bronchoconstriction, vasodilation, increased vascular permeability and recruitment of eosinophils (57). The role and mechanism of PGD₂ has been highlighted in studies involving the DP receptor expressed on Th2 cells, eosinophils and basophils (58).

OVA-sensitized mice deficient in DP receptors were observed to show a decreased in Th₂ cytokines and infiltration of lymphocytes post allergen challenge compared to the wild type counterparts (59). Additionally, studies using Balb/c female mice highlighted that DP receptor activation increases inflammation and eosinophilia in the animal model (60). In a similar study, mice directly treated with PGD₂ showed increased number of eosinophils,

lymphocytes, and macrophages as well as expression of IL-4 and IL-5 when compared to the control group (61). Work done in transgenic mice overexpressing lipocalin-like prostaglandin synthase showed elevated levels of IL-4 and IL-5 as well as increased eosinophilia after allergen challenge (62). High levels of PGD₂ have also been reported in bronchoalveolar lavage fluid of asthmatic patients upon allergen challenge. Interestingly, this causes bronchoconstriction in human asthmatic patients but not in healthy individuals highlighting its role in human allergic asthma (63). These results clearly establish the role of PGD₂ in the inflammatory allergic response.

Leukotrienes—The cysteinyl leukotrienes (LTC₄, LTD₄ and LTE₄) are potent mediators of inflammation whose receptors (CysLT receptors) are found on mast cells, eosinophils, B-lymphocytes and macrophages (64). They mediate bronchoconstriction, increased vascular permeability, eosinophil activation and increased mucus secretion (65). Expression of the CysLT receptor has been found in the lungs of both human and mouse models. LTD₄ is important for mast cell proliferation and cytokine production. Knockdown of CysLT1 receptor significantly decreased the cellular and pathophysiological responses of mast cells in murine models (66). Mice lacking CysLT1 receptors showed decreased Th2 responses and eosinophil infiltration post challenge in the house dust mite (HDM) model (64).

Cysteinyl leukotrienes also play a role in the late phase response by modulating and attracting immune cells, which mediate the process of airway remodeling. Administration of LTE₄ to airways of mice potentiates eosinophilia and goblet cell metaplasia (64). LTE₄ was also found to induce hyper-responsiveness and eosinophilia in human subjects (64, 65)

Recent studies have identified a possible role of LTB₄ in asthma pathogenesis. Its BLT1 receptor is expressed on leukocytes and has been identified on human airway smooth muscle cells (65). BLT1 is believed to be involved in the early phase response and play a role in granulocyte recruitment to the airways. Mice deficient in BLT1 had significantly lower AHR and goblet cell metaplasia than the wild type (64, 65). An increased level of LTB₄ has been found in asthmatics but its role in asthma pathogenesis is still controversial (65).

BAFF—B-cell activating factor (BAFF) is a member of the TNF family and plays a role in the generation and maintenance of mature B-lymphocytes (67). Its role in asthma is still being elucidated and studies to date have yielded conflicting results. In a mouse model of asthma, it was found that BAFF was present in alveolar cells surrounding the bronchi of OVA-induced lung tissue. Increased BAFF transcripts were also detected in splenocytes suggesting its pro-inflammatory effect (68). BAFF levels were also reported to be higher in the serum and bronchoalveolar lavage fluid of asthmatic patients when compared to normal individuals. These increased levels correlated with total number of lymphocytes, neutrophils and eosinophils (69). Based on these results it would seem that antigen induced production of BAFF may contribute to class switch recombination and immunoglobulin synthesis in B-cells. BAFF was also found to increase peripheral blood mononuclear cells in asthmatic patients and T cell-APC conjugate formation (70). Based on these findings, BAFF is believed to play a role in airway hyper-responsiveness (AHR) mediated by T-cells. However, further work is needed to ascertain the mechanism of action and role of BAFF in the human asthmatic response.

Mediators produced by epithelial cells

TSLP—The epithelial cells express thymic stromal lymphopoietin (TSLP) with the highest levels in lungs and skin epithelial cells (71). It was first thought to primarily be a lymphocyte growth factor but it is now clear that TSLP elicits its effects on other cells types, namely DCs, eosinophils, mast cells and ASM cells (72). Studies in both human and murine models have implicated TSLP in the development and progression of allergic diseases. In mice, TSLP has been found to amplify the differentiation of alternatively activated macrophages therefore contributing to allergic inflammation (73). Mice challenged with HDM extract in the absence of TSLP receptor showed drastic reduction in allergic inflammation and pro-inflammatory cytokines with decreased eosinophil recruitment in bronchoalveolar lavage fluid and decreased mucus production (74).

TSLP was also found to stimulate myeloid derived DCs to prime T-cells generating a Th2 response in human derived lung DCs (71). TSLPR deficient DCs had reduced capacity to present antigen to T-cells (72). Studies using human ASM cells showed that TSLP induced migration through STAT3 activation (75) and lung specimen from human asthmatic patients revealed that TSLP-induced senescence was required for airway remodeling *in vitro* (76). TSLP has also been reported to have an effect on IL-10 producing T regulatory cells. Examination of bronchoalveolar lavage fluid from human patients has shown that TSLP inhibits IL-10 producing-Tregs (71). Collectively, these studies suggest that TSLP contributes to the pathogenesis of asthma by enhancing pro-inflammatory Th2 responses and suppressing tolerance to antigens in the lungs.

SP-A and SP-D—Surfactant proteins (SP-A and SP-D) are large hydrophilic proteins, which cover the peripheral airways and play a role in pathogen uptake and phagocytosis. They also provide a protective mechanism during allergen challenge by scavenging allergen, thus, preventing cross linking of response and release of mediators from mast cells (77). The evidence supporting the role of SP-A and SP-D in asthma has been somewhat contradictory; anti-inflammatory or having no effect on inflammation. Studies using an OVA-sensitized and challenged mouse model showed that SP-A aids in maintaining homeostasis of the airways by inhibiting TNF- α secretion from mast cells. SP-A knockout mice exhibited increases in inflammatory cells, mucus production and lung damage when compared to the wild type (78). Mice lacking SP-A were found to have increased inflammation during *Mycoplasma pneumoniae* infections (bacteria that may colonize the airways of patients with chronic asthma) mediated by mast cells. In human patients, SP-A was found to inhibit this effect (79).

Studies linking SP-D with allergic asthma found that SP-D knockout mice showed increased levels of IL-13 in their lungs, which exaggerated the immune response after allergen challenge (80). Conversely, another study showed that SP-D^{-/-} mice had impaired Th2 responses and reduced inflammation after allergen challenge (81). In human patients, SP-D inhibited the chemotaxis of eosinophils suggesting an anti-inflammatory role in the lungs of asthmatic subjects (82). Children with decreased or absent SP-D in bronchoalveolar lavage fluid were found to have more frequent respiratory diseases (83). Most of the data seem to

suggest that both human and murine SP-A/D seem to play a role in controlling allergy and airway inflammation.

Activin A—Activin A belongs to the TGF- β superfamily and plays a role in development and tissue repair. Increasing evidence suggests that this cytokine plays a dual role by both enhancing and suppressing immune response based on the microenvironment and context of the response (84). Studies conducted in mice indicated that there were increased levels of activin A in the bronchoalveolar lavage fluid, which coincided with increased Th2 cytokines. IL-13 has also been reported to increase activin A in bronchoalveolar lavage fluid, with its effects being attenuated by treatment with Fullistatin, suggesting that IL-13 may regulate activin A during allergic inflammation (85). This correlates with human studies, which showed that activin A levels were increased in patients with severe asthma. Isolation of T-cells from severe asthmatic patients showed increased levels of activin A mRNA when compared to normal subjects (86).

Conversely, another study showed that endogenously produced activin A could suppress antigen-specific Th2 response and protected mice against developing airway hyper-responsiveness by induction of T regulatory cells. Adoptive transfer of activin A induced the same effect (87). Similarly, activin A produced by normal human bronchial epithelial cells in response to TNF- α down-regulates further production of TNF- α and IL-13. This suggests that activin A may contribute to the resolution of the inflammatory response in human allergic asthma. (84). Taken together, there is evidence to support the role of activin A as a regulator of events involved in allergic airway inflammation and asthma pathogenesis.

Chronic/Late Phase Response

There is interplay between the innate and adaptive immune cells in asthma. In a nut shell, dendritic cells, which are professional APCs, process and present antigens to T-cells via MHC Class II. DCs are driven to maturation by the production of GM-CSF from epithelial cells as well as the influence of IL-4 from immune cells. (6). The interaction with the DC and the T-cell, as well as the presence of cytokines, leads to a Th2 polarization. Chemokines secreted from mast cells recruit these T-cells, eosinophils, and neutrophils to the airways. Th2 cells prime B-cells to class switch and produce IgE, which further mediates asthma pathogenesis.

Immune Cells

Eosinophils—Eosinophils are associated with many allergic responses including asthma and their accumulation in the lungs is a defining characteristic of asthma in both humans and animal models (88). They develop and differentiate under the influence of primarily IL-5, enter the circulation and traffic to the site of infection or inflammation (28). Activation of eosinophils leads to the release of pro-inflammatory mediators such as major basic protein (MBP), cationic protein, LTC₄, PGE₂, thromboxane and PAF. They also possess the ability to synthesize and release a host of interleukins (IL-3, IL-4, IL-5, IL-8, IL-10, IL-12, IL-13), chemokines (CCL5/RANTES and CCL11/eotaxin-1), TNF- α and TGF- β (89).

Various studies have highlighted that neutralization of IL-5 can suppress pulmonary eosinophilia in response to allergen challenge (90). IL-5 deficient mice as well as mice treated with anti-IL-5 antibodies also showed marked decreases in eosinophil trafficking (91). Studies done in the last few years suggest that eosinophils have the potential to function as APC and augment B-cell activation. Wang et al., showed that wild type Balb/c mice elicited B-cell priming and produced antigen specific IgM post sensitization of antigen free alum. The major cell type that was found in the spleen, up to 6 days post sensitization, was eosinophils. Similar experiments carried out with Balb/c eosinophil deficient mice (Delta dblGATA) failed to produce these results (92). Human clinical studies have confirmed some aspects of these observations particularly airway remodeling and deposition of eosinophil products in tissues of asthmatic patients (93).

Neutrophils—Neutrophils are one of the first inflammatory cells to be recruited to the site of injury or exposure to allergen. Neutrophils are recruited by IL-8, CXCL5, CCL3, LTB₄ and GM-CSF (94). They produce IL-8, TNF- α and TGF- β from their granules and thus contribute to the recruitment of more neutrophils (94, 95). Neutrophils also produce metalloproteases and elastase, which play a role in increased vascular permeability, mucus hypersecretion and bronchoconstriction (94).

The role of neutrophils in the pathogenesis of asthma has been controversial. Studies using mouse models have shown increases in neutrophils in the airways of OVA-sensitized and challenged mice post allergen challenge (96). In human subjects, neutrophils seem to play a more critical role in severe asthma as elevated counts have been found in bronchoalveolar lavage fluids (97). The IL-17 producing cells have been suggested to be involved in the upregulation of IL-8 and subsequent recruitment of neutrophils to the airways (98). Some human patients with severe disease have been reported to have a neutrophil dominated airway inflammation (99, 100). This often correlates to worse outcomes and lower responses to inhaled corticosteroids since these drugs have been found to promote neutrophil survival by suppressing apoptosis and upregulating LTB₄ (101).

Conversely, another human study reported no noticeable change in neutrophil levels between asthmatic and non-asthmatic patients (102). A possible explanation put forward to clarify these findings is that the increase in neutrophils may be due to the fact that airway remodeling provides a suitable environment for neutrophil survival and recruitment, but they are not actively contributing to the pathogenesis observed (103).

Dendritic Cells—DCs are located in the airway epithelium and continuously scan for and sample antigen that the lungs are exposed to. Being professional APCs, DCs are able to traffic to the lymph nodes where they present processed antigens to T-cells. The response is often times protective (Th1 response) but in allergic individuals, the Th2-dominated response is mediated (104).

Evidence of the role of dendritic cells in asthma has been established by work done in murine models as well as human subjects. Studies suggest that there is an increase in DCs in the airway after allergen challenge in OVA sensitized mice (105). Injection of DCs in mice resulted in the activation and recruitment of leukocytes to the airways. Studies in IL-4 and

CD28 knockouts revealed that the production of IL-4 by host cells and co-stimulation of T-cells by DCs were critical to mediating these responses (7). Depletion or inhibition of these cells has also been found to abate hyper-responsiveness in animal models. Lung DCs treated with immunomodulator, Fms-like tyrosine kinase 3 (Flt3) ligand, were found to demonstrate impaired antigen uptake compared to the untreated controls. It was also found that there was a decrease in the production of Th2 cytokines and increased Th1 cytokine and IL-10 (106).

Studies involving asthmatic subjects revealed that treatment of human blood DCs with urban particulate resulted in T-cell priming and production of IL-6 and TNF- α (107). Analysis of peripheral blood and sputum of asthmatic patients showed an increase in myeloid DCs post allergen challenge (108). Taking together, these results demonstrate the role of DCs in antigen presentation, mediation of Th2 polarization and subsequent airway inflammation.

Th2 Cells—Priming of naïve T-cells by DCs after allergen challenge leads to a Th2 response resulting in the production of a host of cytokines. These cytokines promote IgE production from B-cells, eosinophilia and AHR in the airways. Experimental data from animal models have documented the expression of Th2 cytokines (particularly IL-4, IL-5 and IL-13) in the lung, suggesting that these cytokines are critical for the development of hallmark features of asthma. Several human studies have also shown increased levels of these cytokines in bronchoalveolar lavage fluid of asthmatic patients (63) and suppression of Th2 cells has also been found to abrogate characteristic asthmatic symptoms (109).

STAT6, GATA 3 and the Th2 Phenotype—The major cytokine promoting the Th2 fate is IL-4 which binds to the IL-4R (IL-4R α) on naïve CD4⁺ T-cells (IL-13 also activates this pathway). This leads to dimerization of the receptor subunit resulting in the phosphorylation of the tyrosine residues by Janus Kinases (JAK). Cytoplasmic STAT6 docks unto the phosphorylated receptor via the SH2 domain, enabling JAK to phosphorylate STAT6. Phosphorylated STAT6 then dimerizes and is translocated to the nucleus (110). Once in the nucleus, STAT6 can bind to DNA and regulate the transcription of target genes (111, 112).

The role of STAT6 in mediating the Th2 response has been showed both *in vitro* and *in vivo*. STAT6 knockout mice were shown to have significantly reduced Th2 mediated responses such as airway eosinophilia and mucus production when compared to the control group (113). STAT6 also regulates the expression of GATA3, (the Th2 ‘master controller’), which is responsible for the expression of the Th2 cytokines (110). GATA3 stabilizes the Th2 phenotype by, firstly shutting down Th1 development, through repression on the IL-12 receptor, and secondly, by regulation its expression by positive feedback (114). GATA3 can also promote transcription of Th2 cytokines independent of the STAT6 pathway via notch signaling expression (115) and via signaling through STAT5 (116).

Other subsets of T-helper cells

Th17 cells: This subset of IL-17 producing T-cells were originally thought to be CD4⁺ memory cells until it was discovered that they were a distinct subtype now referred to as Th17 cells. Naïve T-cells differentiate into Th17 cells in the presence of IL-6 and TGF- β and through the induction of ROR γ t (retinoic acid-related organ receptor γ t) and suppression of FoxP3 (117). IL-23, secreted by professional APCs, is important for the maintenance of

Th17 cell population. These cells were found to increase AHR and inflammation in the airways of HDM-sensitized and challenged mice, which were decreased with the administration of Fms-like Tyrosine Kinase-3 (Flt3) ligand (118). Th17 cells secrete IL-17 (which has IL-17A and IL-17B are the most important in asthma pathology), IL-22 and also play a role in neutrophil activation and recruitment (119). Evidence of this has been shown in studies using transgenic mice, anti-IL-23 antibodies and adoptive transfer of Th17 cells. Results suggested that IL-23 and Th17 cells induce neutrophil airway inflammation and promoted AHR (120).

Th9 and Th22 cells: Th9 cells develop from Th0 cells in the presence of TGF- β , IL-4 and require transcription factors STAT6, IRF4 (interferon response factor 4), GATA3 and PU1. They secrete IL-9, IL-10 and IL-21, though the roles of the latter two are unclear in Th9 functioning (121). Two studies have made an attempt to test *in vivo* functions of Th9 cells using neutralization and adoptive transfer. It was found that neutralizing IL-9 ameliorated asthma symptoms and that Th9 cells promoted allergic responses by recruiting mast cells to the lungs (122, 123).

Th22 cells are believed to differentiate under the influence of IL-6 and TNF- α (124). Th22 cells produce IL-13, IL-26 and IL-22 (which is the most important). These cells play a role in mucosal immunity with IL-22 receptors found on keratinocytes (125).

NKT cells and $\gamma\delta$ T cells

These two cells types have recently emerged as contributors to the pathogenesis of asthma. NKT cells are a subset of T-cells with some similarities to Natural Killer (NK) cells. They respond to non-peptide antigens presented by non-classical MHC molecule, like CD1d, which leads to the production of both Th1 (IFN- γ) and Th2 (IL-4) cytokines (126). NKT cells have been reported in the lungs after allergen challenge and murine models have demonstrated that they can contribute to the development of AHR but their role in asthma is still controversial (127, 128).

The $\gamma\delta$ T cells are comprised of a gamma (γ) and delta (δ) chain instead of the $\alpha\beta$ chains found on most CD4+ T-cells. They recognize small organic molecules and lipids presented by CD1 (126). Their role in asthma is still unclear as finding from different studies are contradictory, some suggesting an anti-inflammatory effect whereas others suggest a pro-inflammatory effect. It however seems that they may play a role in maintaining lung homeostasis (22).

Chronic or Late Phase Mediators

Th2 Cytokines

IL-4—The contribution of Interleukin (IL)-4 in the development of allergic asthma was discovered in the 1990s and is described as the main cytokine involved in the pathogenesis of allergic responses. Its reported functions include airway remodeling by stimulation of mucus producing cells and fibroblast; inducing B-cells to isotype-switch and produce IgE; and up-regulation of adhesion molecules enabling the migration of leukocytes to the airways (129). Studies using IL-4 knockout mice showed that these mice were protected from

developing sustained airway hyper-reactivity and aspects of airway remodeling (130). Silencing of IL-4 in OVA-sensitized and challenged mice resulted in significant decreases in eosinophilia, AHR and inflammation (131).

IL-5—IL-5 seems to be the major cytokine driving the terminal differentiation, activation and survival of committed eosinophil precursors. The relationship between IL-5 and the development of eosinophilia has been firmly established in IL-5 transgenics, knockout mice as well as mice treated with anti-IL-5 antibodies (132). C57BL/6J mice were shown to have increased IL-5 and eosinophil infiltration in their lungs, which was not observed in the IL-5 deficient of knockout mice (133). In human studies major target of IL-5 is eosinophils and it has been found to prolong eosinophil survival and enhance its effector functions, thus contributing to the pathogenesis seen in late phase asthmatic responses (134, 135).

IL-13—IL-13 has similar biological properties and activities to IL-4. This is partly due to the structure of their receptors; IL-3 binds to the alpha chain of the IL-4 receptor (136). The functions of IL-13 in asthma have been demonstrated using *in vivo* animal models as well as biopsies and culture of human cells. Allergen challenged IL-13 deficient mice failed to develop AHR or significant mucus changes despite the continued expression of IL-4 and IL-5. Reconstitution with recombinant IL-13 restored characteristic pathology (9, 137). These observations help to clarify the ongoing debate of the roles of IL-4 and IL-13. They suggest that IL-13 alone may be contributing to the main physiological consequences of the disease whereas IL-4 generates the initial Th2 response (138). IL-13 has also been shown to independently elicit many key pathological features of asthma including migration and upregulation of adhesion molecules, goblet cell hyperplasia and stimulation of airway hyperresponsiveness (139).

Recent studies have indeed highlighted the role of IL-13 in the generation and persistence of airway inflammation and remodeling using anti-IL-13 monoclonal antibodies. Mice that were challenged after treatment with mAbs were observed to have significantly inhibited the generation and maintenance of chronic airway inflammation (140). Segmented allergen challenge studies in mild allergic asthmatics demonstrate an increase in IL-13 protein and mRNA in bronchoalveolar lavage fluid. Clinical trials with anti- IL-13 antibodies (Tralokinumab and Lebrikizumab) revealed important insights into the role of IL-13 in human asthma. Inhibition of IL-13 resulted in improvement pulmonary function which has been linked to the attenuated effect of IL-13 inhibition on airway smooth muscle cells (141). In other studies, neutralizing mAbs block IL-13 activity and inhibit the interaction between IL-13 and its receptors. These mAbs were also able to significantly attenuate allergen-induced bronchoconstriction (142). Although results from clinical trials have been promising, there is still the need for future studies to determine the full-scale of activities of these treatments as well as the effects on different asthmatic phenotypes.

Th2 cytokines in human asthma

Increased levels of all the Th2 cytokines have been shown in the airways of patients with asthma. Bronchial biopsies and sputum show increased levels of both mRNA expression and protein levels of IL-4, IL-5, IL-13 and their receptors (132). IL-4 and IL-13 has been found

to disrupt the human airway epithelial barrier that may contribute to airway inflammation in allergic asthma (143).

IL-17

IL-17 acts on epithelial, endothelial and hematopoietic cells and can induce the expression of pro-inflammatory cytokines (144). The role of IL-17 in prompting inflammation by recruiting neutrophils has been shown by both animal models and human biopsies with neutralization of IL-17 greatly reduced pulmonary neutrophilia and overexpression results in enhanced recruitment of neutrophils (145). In a chronic model of asthma, Balb/c mice deficient in IL-17R showed no changes in airway inflammation however mucus producing cells were decreased, suggesting IL-17 may play a role in goblet cell hyperplasia in the airways (146). Similar findings were observed in serum and sputum from patients with asthma after 24 hours of bronchial challenge highlighting the role of Th17 cells and IL-17 in the late phase response (147). Studies involving both human asthmatics and mouse models showed that Th17 and IL-17 levels were higher in peripheral blood (humans) and splenocytes (mouse) of asthmatics than in healthy controls (148).

IL-9

IL-9 exerts its effects on a number of cells but its main effector function is promoting development and proper functioning of mast cells (149). In a chronic model of asthma, IL-9 was shown to enhance mast cell numbers in the lungs, which greatly contributed to chronic airway inflammation. Mice treated with anti-IL-9 antibodies were protected from these responses (150). OVA-sensitized and challenged mice, treated with anti-IL-9 antibodies, also demonstrated inhibition of cytokine production, particularly IL-17 in a chronic model of asthma (151).

IL-9 also plays a role in B-cell development and function. It enhances IL-4 mediated IgE and IgG production in B-cells (152). Airway epithelial and smooth muscle cells are also affected by IL-9 (149). Increased levels of IL-9 mRNA were found in bronchoalveolar lavage fluid of asthmatic patients and IL-9 was enhanced during asthmatic inflammation (153). Its specific role in asthma is still being elucidated and there is still much debate as to whether its production in human asthmatic cases is by Th9 cells.

IL-22

IL-22 is thought to play both a pro-inflammatory and anti-inflammatory role. In Balb/c mice, IL-22 (by an IL-10 associated mechanism) was found to suppress eosinophil inflammation of the airways (154). Studies using IL-22 deficient mice and anti-IL-22 antibodies suggest that IL-22 may be required for early onset of asthma but not in its progression (155). In humans, *in vitro* studies have shown that IL-22 inhibited secretion of IFN- γ , which decreased production of pro-inflammatory chemokines (RANTES and CCL10) as well as decreased T-cell cytotoxicity (156). Another study however reported that there was no difference between IL-22 levels in asthmatic patients when compared to normal subjects (157).

IL-21

IL-21 is produced by activated CD4 T-cells, specifically follicular T helper cells (Th_f) and Th17 cells, and has been found to co-stimulate B and T-cell proliferation and regulation of NK cell activation and expansion. It regulates T-cell proliferation and differentiation as well as normal humoral responses (158). The use of transgenic mice has showed the importance of IL-21 in the regulation and production of antibodies. Results suggest that IL-21 is critical for maintaining low IgE levels under both physiological and pathological conditions in murine asthma models (159). Another study showed increased levels of IL-21 post allergen challenge in a chronic model of asthma, suggesting that IL-21 may play a role in remodeling (160). IL-21 drives inflammation by promoting the expansion and survival of lymphocytes. It has been suggested that IL-21 is able to inhibit the induction of Foxp3 T regulatory cells as well as IL-10 producing Tregs and B-cells (161). Much of the available data on IL-21 in allergic asthma has been collected primarily from work done using mouse models. Its role in the pathogenesis of human allergic asthma needs to be assessed.

IL-33

Many cell types following pro-inflammatory stimulation express IL-33. It binds to its heterodimeric receptor (ST2 and IL-1R accessory proteins), which promotes signaling via the TIR domain of IL-1RAP and activates several signal proteins including NF κ B, p38 and JNK (125). IL-33 has been found to drive development of Th2 cells to produce IL-5 in asthmatic mice and similar results have been observed in *in vitro* cultures of human CD4⁺ cells (162). Blockade of IL-33 was found to ameliorate airway inflammation of OVA-sensitized and challenged mice as well as decreased Th2 cytokine levels and eosinophil infiltration in bronchoalveolar lavage fluid (163).

Examination of IL-33 secretion in mast cells suggest that bone marrow derived mast cells are capable of producing and serving as endogenous sources of IL-33 and IL-33 plays a major role in regulation of mast cell functions (164). Analysis of alveolar macrophages in a Balb/c chronic model of asthma showed that there were increased levels of mRNA expression and immunoreactivity of IL-33 in the airways of mice post allergen challenge (165). These results suggest that IL-33 drives activation of alveolar macrophages and has an important role in the pathogenesis of airway inflammation.

Recent evidence suggests that IL-33 has an effect on dendritic cells and goblet cell. Tanabe et al, showed that IL-33 stimulated apical IL-8 release from goblet cells promoting enhanced airway inflammation and goblet cell metaplasia. Neutralization with anti-ST2 antibody attenuated these responses (166). Endobronchial biopsy studies revealed an association with IL-33 and severe asthma with IL-33 expression being much higher in ASM of asthmatic patients when compared to the control groups (125). The data collectively suggests that IL-33 plays a role in the pathogenesis of asthma.

TGF- β

Transforming Growth Factor- β is a multifunctional cytokine and exists in three isoforms (TGF- β 1, TGF- β 2 and TGF- β 3) with an array of effects on various cells (167). TGF- β 1 binds to its receptor (T β R-II), which recruits T β R-I leading to activation of Smad2 and

Smad3. The phosphorylated Smads are then translocated to the nucleus where they elicit their effect on gene expression (168). TGF- β is secreted by both structural and immune cells and is involved in inflammation and remodeling. It has been implicated as a pro-inflammatory cytokine, which elicits its effects primarily on structural cells contributing to the propagation of the inflammatory response (169).

Work done using transgenic mice has substantiated the positive association between TGF- β and ASM enlargement. Deletion or prevention of TGF- β expression has been shown to reduce ASM enlargement (168). Other roles of TGF- β family members include chemotaxis of inflammatory cells (specifically mast cells), regulation of the immune response, inhibition of the proliferation of many cell types and stimulation of ECM proteins (125, 167). TGF- β also plays a role in airway remodeling, epithelial changes, sub-epithelial fibrosis and ASM remodeling which has been corroborated by various *in vitro* and *in vivo* studies using both animal models and human subjects (125). Family members also play a regulatory role in the immune response. TH3 regulatory cells secrete high level of TGF- β , which suppresses both Th1 and Th2 responses (125, 167).

TGF- β has been implicated in the proliferation of ASM in human subjects. Its expression was higher in patients with mild to severe asthma when compared to the control groups (168). Increased TGF- β levels were also found in bronchoalveolar lavage of asthmatic patients and has been attributed to the increases in macrophage and eosinophil infiltration (169). Although there is evidence to suggest that TGF- β plays a role in pathogenesis of human allergic asthma, the issue regarding its exact role is still controversial. There is also by far more evidence documenting its role in animal models than in human subjects. The role of TGF- β in human allergic asthma still needs further elucidation.

Chemokines

The role of over 40 different chemokines and their receptors in allergic asthma has largely come from extensive work in animal models. Several CC-chemokines are responsible for the initiation and maintenance of airway inflammation in asthma and contribute to the pathogenesis of airway remodeling (63). The role of chemokines is to serve as chemotactic signals, activate adhesion molecules (specifically the integrins), which enables adhesion and diapedesis of leukocytes and direct cells to where they are needed (135). However, dysregulated recruitment of immune cells to peripheral tissue leads to chronic disease and irreversible tissue damage (170).

Chemokines and Structural Cells—Structural cells like fibroblast, epithelial and endothelial cells have been shown to secrete chemokines. Activation of chemokines in epithelial cells dictate the type of cells that will be recruited and therefore play a role in the inflammatory response in the lungs (170, 171). Activation of epithelial cells by IL-4 or IL-13 leads to the production of chemokines that recruit Th2 cells and eosinophils (171).

Chemokines and Immune Cells—Mast cells secrete chemokines, namely CCL2, CCL3 and CCL11, which are involved in the attraction of leukocytes to the airways. Studies have suggested that after activation, mast cells can mobilize their CCR3 to the surface resulting in the amplification of IL-13 production (172, 173). These findings allude to the impression

that mast cells may also be involved in the late phase responses. LTB₄ receptor, BLT₁, is expressed on a host of immune cells and can induce chemotaxis and adhesion of these cells. BLT₁ is required for the initial entry of Th2 cells into the airways. Absence of this receptor has been found to decrease inflammation and AHR (135).

Th2 cell recruitment is further amplified by the release of IL-4, IL-5 and IL-13 and upregulates Th2 chemokines CCL17 and CCL22 as well as eosinophils chemokine CCL11 and CC24 to the lungs (22, 135). Chemokines have also been shown to promote polarization of T-cells and enhancing the production of Th2 cytokines. Neutralizing CXCR4 by antibodies has been shown to reduce AHR and eosinophilia in transgenic mice (174). Human studies suggest that CCR4 and its ligand are increased in peripheral blood and play a role in recruiting Th2 cells to the airways of asthmatic individuals (175).

Dendritic cells are influenced by the chemokines secreted by epithelial cells in close proximity to play a role in their differentiation, maturation and recruitment. As a DC matures its chemokine receptors changes; precursor DC expresses CCR2 and CXCR4, an immature DC expresses CCR1, CCR2, CCR5, CCR6, CXCR2, and CXCR4 whereas a mature DC expresses a single receptor, CCR7 (176). The change in the chemokine receptor facilitates trafficking of the DC from site of antigen uptake to lymph node, allowing successful antigen presentation to T-cells.

Regulatory Mediators

Tregs

T regulatory cells are important for the induction and maintenance of immunological tolerance to foreign and self-antigens. Natural T regulatory cells (nTregs) express CD4+CD25+ and transcription factor Foxp3, which is critical for their development (177). The nTregs develop in the thymus under signals that are different from non-regulatory T-cells and function by cell-to-cell contact; as opposed to cytokine secretion by Th3 and TR1 regulatory cells (178). TR1 cells are described as antigen specific T regs that are CD25- and produce IL-10 (179).

Several *in vitro* and *in vivo* studies have suggested that both nTregs and IL-10 secreting Tregs play a role in suppressing Th2 responses. Peripheral blood collected from asthmatic showed decreased expression of CD4+CD25+ than in non-asthmatics (126). Other studies highlighted that Tregs were decreased in samples collected from the airways of atopic individuals (180). Shi et al., also observed that patients with moderate to severe asthma showed decreased number of CD4+CD25+ Tregs and had increased Th2 responses (181).

Studies using Foxp3/PMX retrovirus administered intratracheally followed by allergen challenge showed that expression of Foxp3 reduced airway AHR, mucus production and infiltration of inflammatory cells. Reduced levels of IL-4, IL-13 and IL-17 were also reported (182). The nTregs and IL-10 producing Tregs have also been shown to decrease airway inflammation. Adoptive transfer of these cells to CRA-sensitized and challenged mice were also found to decrease Th2 cytokine levels in bronchoalveolar lavage fluid and

inhibit AHR in this model (183). Taken together, all the above data undoubtedly proves the role of Tregs in suppressing and controlling inflammation in asthma.

IL-10

IL-10 is regarded as an anti-inflammatory cytokine which is secreted by TR1 cells. Its receptor, IL-10R, is expressed on many myeloid and lymphoid cells (184). IL-10 induces anergy in an autocrine manner by inhibition of CD28 co-stimulatory signal. It also has anti-inflammatory effects on basophils, mast cells and eosinophils (185).

The functions of IL-10 have been demonstrated by various studies. IL-10 was shown to decrease neutrophil infiltration on murine models of allergic asthma. Blockade of IL-10 resulted in increased IL-1 β and the CXC Chemokines (96). Asthmatic patients have also been reported to decrease levels of IL-10 and TGF- β suggesting the mechanism driving the production of these cytokines may be defected (186). Adoptive transfer of TR1 cells were also found to decrease Ag-specific IgE with IL-10 treated DCs skewed towards a tolerogenic phenotype (187)

Th1 cells

The Th1/Th2 paradigm has been described over 20 years ago and allergic diseases are a result of a tip in the balance towards a Th2 response. There is now increasing evidence to suggest that Th1 cells are somehow involved in this event as well but their exact contribution to the pathogenesis of asthma is still unclear (22). Th1 cells produce IFN- γ , IL-2, TNF- α and lymphotoxin to antagonize Th2 development (126). Th1 master controller transcription factor, T-bet, contribute to Th1 polarization and secretion of IFN- γ (188). Studies have reported that T-bet expression was reduced in asthmatic patients. Knockout mice were shown to have severely diminished Th1 responses and as a result were more susceptible to Th2 type diseases (189).

Conclusion and Future Directions

The work done using animal models has contributed immensely to the growing knowledge and understanding of the pathogenesis of allergic asthma. Transgenic models have enabled researchers to manipulate genes, which have provided new insights on previously unknown mechanisms. Recent research has highlighted that asthma is not only a Th2 response but involves a host of T-helper subsets with pro and anti-inflammatory properties. Emerging mediators such as innate lymphoid cells and NKT-cells have contributed to the heterogeneity of allergic inflammation and shows that innate immunity profoundly shapes the development of asthma. Cells with regulatory potentials namely Tregs and γ/δ T-cells are amenable targets for asthma therapy though further research is needed to ascertain the underlying mechanisms and pathways that generate these inhibitory effects.

The focus of asthma research over the past two decades has been on identification of cells, mediators and pathways involved in airway inflammation. Studies from animal models have highlighted many mediators as potential targets for therapeutic intervention. The development of immune based therapy generated much hope but unfortunately this has not

been successfully translated to better treatment options for asthmatics, and there is still no cure for the disease.

Knowledge regarding the interaction of both arms of immunity and structural cells in contributing to chronic inflammation is still growing. However, the disparity between morphology and functional aspect of the current murine models and human is a hindrance to advance our knowledge on the underlying mechanisms involved in chronic allergic asthma. Current models do not effectively model asthma exacerbations, which account for most asthma related visits to the emergency room. There is, therefore, a need for the development of better models, which can more comprehensively elucidate these mechanisms. Better understanding of this multifaceted disease will no doubt improve our ability to effectively deal with this growing epidemic.

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Highlights

- Asthma involves all T-helper subsets with pro and anti-inflammatory properties.
- Innate immunity profoundly shapes the development of asthma.
- ILCs and NKT-cells contribute to the heterogeneity of allergic inflammation.
- Interaction between immune and structural cells dictates chronicity of asthma.
- Potential targets for therapeutic interventions are highlighted.

Figure 1.

Acute/Early Phase Allergic Response: Crosslinking of the FcεR on previously sensitized mast cells by allergen in the lungs leads to their activation and degranulation. Mast Cells release potent mediators including histamine, cysteine leukotrienes and prostaglandins which are responsible for acute inflammation in allergic asthma as well as laying the foundation for chronic inflammation. Basophils are also activated and release similar mediators to enhance this effect. The airway epithelium also participates in the acute response by releasing TSLP (which acts on mast cells), BAFF (which promotes B-cells to undergo class switch recombination, differentiate into plasma cells and produce IgE); Activin-A (which inhibits further TNF-α/IL-13 production from ASM cells), SP-A/SP-D (scavenges allergen preventing crosslinking of Fc receptors and subsequent degranulation of mast cells), and IL-33/IL-25 (which promotes type 2 ILCs to produce IL-13). ILC2- Innate Lymphoid Cells-Type 2, TSLP - Thymic Stromal Lymphopoietin, PGD2 - Prostaglandin D2, LTB4 - Leukotriene B4, BAFF - B-cell Activating Factor, Baso – basophil, SP - Surfactant Protein, ASM - Airway Smooth Muscle, TNF - Tumor Necrosis Factor.

Figure 2.

Late phase mediators interact to promote chronic inflammation in allergic asthma. Dendritic cells constantly survey the airways, process and present antigens to naïve T-cells (Th0). This primes Th0 cells to develop into Th2 cells under the influence of IL-4. Th2 cells produce cytokines which targets other immune cells (IL-4/IL-13 targets B-cells to isotype switch; IL-5 recruits and activates eosinophils). Other T helper subsets (Th9 and Th17) are also involved in the late phase response and secrete various cytokines and chemokines which are involved in the recruitment of inflammatory cells. This drives chronic inflammation in the lungs and result in airway remodeling and disease pathogenesis. Mediators released from NKT-cells and γ/δ T-cells may or may not play anti or pro-inflammatory roles in asthma pathogenesis

IL – Interleukin, Th – T helper, DC – Dendritic cells, Neutro – Neutrophil, MBP – Major Basic Protein, ECP – Eosinophil Cationic Protein, IFN – Interferon, NKT – NK T-cells, γ/δ T – gamma/delta T-cells, AHR – Airway Hyper-responsiveness, LT – Leukotriene, TSLP – Thymic Stromal Lymphopoietin

Figure 3.

Subsets of T-lymphocytes in allergic airway inflammation and asthma: Naïve T-cells differentiate into different subsets of T-cells based on their transcription factors and cytokines in the environment. Natural regulatory T-cells (T-regs) and IL-10 producing regulatory T-cells (TR1) have the capability to suppress T helper cell as well as mast cell, basophils and eosinophil functions, thus decreasing the chronic inflammatory response in allergic asthma.

Th - T-helper, TGF - Transforming Growth Factor, IL – Interleukin, T-bet - T- box expressed in T-cells, ROR - retinoic acid-related organ receptor, Foxp3 - forkhead box p3, TNF - Tumor Necrosis Factor, DC - Dendritic cell.

Table 1

Comparative analysis of animal models of allergic asthma with features of human allergic asthma

Animals	Similarities	Differences	References
Rats	IgE is the primary anaphylactic antibody	Tolerance with increased sensitization	14
	Generate EPR and LPR Generate AHR Goblet cell hyperplasia	Low bronchoconstriction	15
Guinea Pigs	Lung anatomy Generate EPR and LPR Airway remodeling	IgG ₁ is the primary antibody AHR absent No eosinophilia	14, 19081, 19182
Mice	IgE is the primary anaphylactic antibody AHR Airway remodeling Goblet cell hyperplasia	AHR not spontaneous Lung anatomy No late phase response	13, 14, 1094
Rabbits	IgE is the primary anaphylactic antibody Lung anatomy Generate both EPR and LPR	Neonatal immunization required for LPR Eosinophilia absent AHR absent	13, 19283

AHR – Airway hyper-responsiveness EPR – Early Phase Response LPR – Late Phase Response

Table 2

Sensitization and challenge protocol for different strains of murine model of allergic asthma

Strains	Developed Protocols	Observations	References
Balb/c Female	<u>Acute models</u> Sensitization with OVA/alum (i.p.) on Day 1 and 14 Challenged with 1% OVA (Day 28–30) and 5% OVA (Day 32 and Day 44) <u>Chronic Models</u> Sensitization with OVA/alum (i.p.) on Day 0 & 14 Challenge with 1% OVA (Day 28–30) and 5% OVA (Day 32 and 44) Challenge with 1% OVA (Days 52–77) and 5% OVA (Day 79)	High IgE AHR Bronchospasm Goblet cell hyperplasia Airway remodeling Collagen deposition Epithelial cell hypertrophy	13, 18 19384
Balb/c Male	<u>Acute Model</u> Sensitized with OVA/Al(OH) ₃ (i.p.) on Day 0 & 5 Challenge on Day 17	Elevated IgE and eosinophilia AHR	19485
A/J Male	<u>Acute Model</u> Sensitization with Der f 1 and Bla g 2 on Day 0 & 7 Challenge with with Der f 1 and/or Bla g 2 on Day 14 (orotracheally)	Increased eosinophilia AHR (Bla g 2 only)	19586
A/J Female	<u>Chronic Model</u> Sensitization with OVA/alum (i.p.) on Day 0 & 10 Challenge on Days 20 and 21 Repeat exposure to allergen for 12 weeks	Fibrosis and eosinophilia	19687
C57BL/6 Male	<u>Acute Model</u> Sensitization with Der f2 (i.p.) on Days 1 & 14 Challenge on Days 28–30 (intranasally with 0.1% Der f 2)	Decreased levels of eosinophilia Specific IgE AHR	19788

Der f (*Dermatophagoides farinea*) – House Dust Mite; Bla g (*Blattella germanica*)- Cockroach allergen; OVA (Ovalbumin)