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Janus Kinase-3 Dependent Inflammatory Responses in Allergic Asthma

Rama Malaviya^a, Debra L. Laskin^a, and Ravi Malaviya^b

^aDepartment of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ 08854, USA

^bInflammation & Pulmonary Diseases Team, Johnson & Johnson Pharmaceutical Research and Development, Springhouse, PA 19477, USA

Summary

Allergic asthma is a chronic inflammatory condition of the lung characterized by reversible airway obstruction, high serum immunoglobulin (Ig) E levels, and chronic airway inflammation. A number of cells including mast cells, T-cells, macrophages and dendritic cells play a role in the pathogenesis of the disease. Janus Kinase (JAK) –3, a nonreceptor protein tyrosine kinase, traditionally known to mediate cytokine signaling, also regulates functional responses of these cells. In this review the role of JAK-3 in regulating various pathogenic processes in allergic asthma is discussed. We propose that targeting JAK-3 is a rationale approach to control the inflammatory responses of multiple cell types responsible for the pathogenesis of allergic asthma.

Keywords

Janus kinase; Allergy; Asthma; lung inflammation

Introduction

Despite the availability of newer and better pharmacological approaches and cutting edge patient care, the prevalence and severity of asthma continue to rise [1]. According to the National Institutes of Health, an estimated seventeen million people suffer from this devastating disease in the United States alone [2]. Although death is not a common consequence of asthma, it is a cause of severe morbidity. Additionally, frequent emergency room visits owing to acute symptoms associated with exacerbation of asthma contribute significantly to the economic burden of the disease. Thus there is an urgent need to develop novel approaches for its treatment. A better understanding of the pathogenesis of asthma and

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Address correspondence and reprint requests to: Rama Malaviya, Ph.D., Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Rutgers University, 160 Frelinghuysen Road, Piscataway, NJ 08854-8020, USA; Phone 1-732-445-4702; malaviya@rci.rutgers.edu.

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the role of various biochemical signaling molecules in the disease pathogenesis is essential to develop more efficacious target-specific therapeutics.

Asthma is a complex inflammatory condition characterized by reversible airway obstruction, high serum IgE levels, and chronic airway inflammation and remodeling [3–5]. The pathogenic process involves an early phase reaction followed by a late phase reaction, involving coordinated activities of CD4-positive T cells, mast cells, dendritic cells, macrophages and eosinophils [6]. These cells release a diverse array of immunoregulatory mediators thought to contribute to asthma including histamine (mast cells), arachidonic acid metabolites (mast cells, macrophages and eosinophils), proteases (mast cells, macrophages and eosinophils), and various cytokines and chemokines (T cells, mast cells, macrophages and eosinophils), in particular interleukin (IL) –4, IL-5, IL-13, IL-15, IL-17, IL-18, tumor necrosis factor (TNF) α , monocyte chemotactic protein (MCP)-1, MCP-3, MCP-4, macrophage inflammatory protein (MIP)-1 α , IL-8, and eotaxin [7, 8]. The biological/pathogenic effects of the key mediators involved in asthma are listed in Table 1.

Current strategies for the treatment of asthma are mostly based on relieving the symptoms of the disease, or inhibiting the release or activity of pathogenic mediators using β -agonists, steroids, cromolyn sodium, or leukotriene (LT) antagonists. However, the side effects due to chronic usage of these drugs, coupled with their inability to adequately relieve symptoms, has limited their usefulness, and led to a search for effective mechanism-based therapeutics such as antibodies to IL-4 and IL-5 [9, 10], keliximab, a chimeric monoclonal antibody to CD4 [11], omalizumab, a humanized monoclonal anti-IgE antibody [12, 13], and small molecule LT inhibitors [14]. The response to these drugs has been mixed, with some patients showing positive clinical outcome, whereas others remain unresponsive or become refractory to the treatment. This is most likely due to the heterogeneity of the disease and/or variations in patient genetics. Moreover, targeting a single cell type or mediator may not be an optimal approach for the treatment of asthma because of the redundant and overlapping responses of inflammatory cells (Table 1). A more plausible approach may be to target a common upstream event that controls the pathogenic responses of multiple inflammatory components. One such molecule is JAK-3 which has been reported to play role in the pathogenesis of asthma [15–18]. JAK-3 is a non-receptor protein tyrosine kinase (PTK) belonging to the JAK-PTK family, which also includes JAK-1, JAK-2, and TYK-2 [19]. JAKs bind to their transmembrane cytokine receptors through an amino-terminus 4.1, ezrin, radixin, moesin (FERM) comprising JAK homology (JH) domains JH6 and JH7. Kinase activity of JAKs is regulated by FERM and a unique pseudokinase domain, JH2, located immediately following, and towards the amino-terminal site of the catalytically active carboxy terminus kinase domain JH1 [19]. Whereas most members of JAK family are ubiquitously expressed, JAK-3 expression is restricted to the cells of myeloid and lymphoid lineage [20]. Findings that B-cell and T-cell activities are impaired in JAK-3-deficient mice demonstrate that JAK-3 is important in the development and function of these cells [21]. This is supported by the observation that JAK-3-deficient patients develop severe combined immunodeficiency [22, 23].

JAK-3 regulates cytokine signaling by associating with the common γ chain (γ c) of cytokine receptors shared by IL-2, IL-4, IL-7, IL-9, and IL-15. Following ligand binding JAK-3 is

phosphorylated leading to activation and dimerization of the transcription factor Signal Transducers and Activator of Transcription (STAT), and activation of target genes (Fig. 1). Whereas the role of JAK-3 in regulating cytokine signaling in T cells is well established [24], its involvement in non-cytokine signaling is unclear. JAK-3 has been reported to regulate platelet activation and aggregation [25], macrophage expression of inducible nitric oxide synthase (iNOS) and nitric oxide production [26], and mast cell proliferation and functions [17, 27–30]. Accumulating evidence suggests that JAK-3 is also involved in IgE class switching and in mediating responses of dendritic cells, macrophages and cells of non-hematopoietic lineage in asthma. In this review recent findings implicating JAK-3 in the pathogenesis of asthma are discussed (see Figure 2). A better understanding of the molecular mechanisms mediating asthma may facilitate the development of more efficacious approaches to its prevention and/or treatment.

Role of JAK-3 in Mast Cell Responses

It is well established that mast cells play significant role in the pathogenesis of allergic diseases including asthma [8, 31, 32]. Following IgE receptor (FcεRI) crosslinking by monomeric IgE, mast cells release a variety of pro-inflammatory mediators including histamine, serotonin, TNFα, tryptase, chymase, cathepsin G, LTs, prostaglandins (PGs), and platelet activating factor (PAF), as well as various cytokines and chemokines [33, 34]. Since histamine, proteases and TNFα are presynthesized, granule-associated molecules, these mediators are immediately released following mast cell stimulation [33]. In contrast, arachidonic acid metabolites, such as LTC₄ and PGD₂, which must be biochemically synthesized, are released within minutes of cellular activation [35, 36], while cytokines (IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, and IL-13) and chemokines (IL-8, MCP-1, MIP-1α,) which require gene transcription are released hours after mast cell activation [37]. Thus, mast cells play a dual role in asthma; whereas early in the pathogenic response they release presynthesized mediators, later in inflammatory responses, they generate cytokines and chemokines [38, 39]. Together these inflammatory mediators induce the pathogenic, as well as clinical signs and symptoms of the disease. For example, histamine and tryptase are highly spasmogenic to the airway smooth muscles, and together with their mitogenic activity, these molecules induce both hyperplasia and hypertrophy [32]. TNFα and LTB₄ are responsible for the recruitment of leukocytes to sites of inflammation [33, 40, 41]. LTC₄ is a strong inducer of bronchoconstriction and mucus secretion in the lungs [42]. PGD₂ causes vasodilation, increases capillary permeability, and induces mucus production and bronchoconstriction [43, 44]. Mast cell-derived tryptase and chymase not only facilitate leukocyte infiltration, but also regulate the chronicity of the disease by interacting with neighboring airway smooth muscle cells, epithelial and endothelial cells [45–48]. IL-4 and IL-13 have been shown to regulate IgE synthesis, as well as structural changes in the lung [34]. Like other mast cell-derived cytokines and chemokines, these mediators not only recruit leukocytes (neutrophils, monocytes, T cells, and eosinophils) into the lung, but also regulate the biological responses of these cells [37, 49, 50].

The release of mast cell mediators is tightly regulated, and highly dependent on activation and phosphorylation of a number of signaling molecules that sequentially transmit signals from IgE receptors to secretory granules or to the nucleus [51]. The IgE receptor is a

heterotetramer with α and β chains, and a dimer of two γ chains [51, 52]. Both β - and γ -subunits of the IgE receptor/Fc ϵ RI contain immunoreceptor tyrosine-based activation motifs (ITAMs), which allow interaction with protein tyrosine kinases and their substrates via their SH2 domains. The engagement of IgE receptors by antigen triggers a cascade of biochemical signal transduction events, including activation of multiple protein tyrosine kinases [51, 53]. Activation of FYN, SYK, BTK, and JAK-3 tyrosine kinases has been shown to correlate with mast cell mediator release [27, 51, 54].

The observation that mast cells abundantly express JAK-3 in their cytoplasm prompted a number of studies aimed at exploring a possible role of the JAK-STAT pathway in mast cell functioning [30, 55–57]. JAK-3 deficient mice have been reported to possess significantly fewer T cells relative to wild-type controls [21, 58]. Since murine mucosal mast cells (i.e., mast cells of the lungs, gut and peritoneum) depend on T cell-derived cytokines like IL-3, IL-4 and IL-9 for proliferation and growth [59–61], it was suggested that JAK-3 deficiency would result in impaired proliferative responses of mast cells. In support of this hypothesis, Irani and colleagues [62] demonstrated that tryptase-positive mast cells are absent in the gut of patients with defective T cell functions, such as AIDS. Suzuki and coauthors [30] also reported significantly fewer mast cells in the peritoneum of JAK-3 deficient mice when compared to wild-type mice. In contrast, further analyses of mast cells in the peritoneum, skin, urinary bladder and lungs of JAK-3-deficient mice and wild-type mice revealed identical number and staining characteristics [55]. Moreover, when mast cells cultured from bone marrow cells of JAK-3-deficient mice were injected into the peritoneum of mast cell-deficient mice, JAK-3-deficient mast cells differentiated and matured normally and acquired organ-specific phenotype [55] suggesting that JAK-3 may not affect maturation or phenotypic differentiation of mast cells *in vivo*.

IgE-receptor mediated activation of mast cells has been widely utilized to study signaling events in this process. In RBL-2H3 cells, a rat mast cell line, JAK-3 is readily activated and phosphorylated following IgE-receptor cross linking, a process blocked by a quinazoline-derived (4-(4'-hydroxylphenyl)-amino-6, 7-dimethoxyquinazoline) pharmacological inhibitor of JAK-3 [28]. Spleen tyrosine kinase, commonly known as SYK, is known to mediate Fc receptor signaling and plays a key role in mast cell activation and degranulation [63]. Evidence also suggests a role of JAK-3 in the fine-tuning of IgE-mediated SYK-dependent signaling in mast cells [28]. More extensive studies are required to fully understand the relationship between JAK-3 and SYK activation in Fc ϵ RI - dependent signaling in mast cells.

Primary cultures of mast cells can be established *in vitro* by culturing bone marrow precursor cells with IL-3 [64]. These bone marrow derived mast cells (BMMC) provide a useful model to investigate the physiological role of specific genes in mast cell responses. Mast cells cultures from the bone marrow of JAK-3-knockout mice (JAK-3-deficient BMMC) were found to release substantially reduced amounts of histamine and LTC₄ in response to IgE/antigen challenge when compared to BMMC from wild-type mice [27]. The observation that the effects of JAK-3 deficiency were more pronounced on the release of LTC₄ suggests impairment in arachidonic acid release or metabolism. Since LTC₄ contributes to several pathobiological responses in asthma (e.g. bronchial smooth muscle

constriction, submucosal edema, increased mucus secretion etc.), reduced release of LTC₄ through targeting of JAK-3 in asthma may prove to be therapeutically beneficial. This is supported by findings that LT synthesis inhibitors, LT-receptor antagonists or modulators of 5-lipoxygenase pathway are effective in alleviating clinical symptoms of asthma [65]. Interestingly, expression of IgE receptors on wild type and JAK-3-deficient BMMC was found to be comparable indicating that reduced responsiveness of JAK-3-deficient BMMC was not due to alterations in IgE receptor expression [27]. Treatment of mouse BMMC or rat RBL-2H3 mast cells with JAK-3 inhibitors also resulted in substantially reduced amounts of β -hexosaminidase (a mast cell granule associated enzyme), LTC₄ and TNF α release after challenge through high affinity IgE receptors [28, 29]. Notably, human mast cells exhibited similar responses when treated with a JAK-3 inhibitor [28]. In human mast cells, JAK-3 inhibition significantly blocked the release of IgE/antigen-induced β -tryptase and LTC₄. These studies clearly demonstrate that JAK-3 plays an important role in IgE receptor-mediated mast cell responses and that targeting JAK-3 may help to alleviate or substantially reduce allergic and inflammatory responses of the lung.

When sensitized and challenged with ovalbumin (OVA), severe peribronchial and perivascular infiltration of eosinophils is observed in lungs of wild-type mice [66]. In these mice OVA also induces proliferation of goblet cells, a marker of increased mucus secretion in the airway epithelium. These effects are not observed in JAK-3-deficient mice [17]. Similar findings are observed using a pharmacological inhibitor of JAK-3. OVA-challenge is also known to induce airway hyper-responsiveness. Treatment of OVA-sensitized and OVA-challenged wild-type mice with a JAK-3 inhibitor prevented methacholine-induced airway hyper-responsiveness and influx of eosinophils into the airway lumen. Consistent with these findings, Kudlacz et al. [16] have reported that a small molecule inhibitor of JAK-3 markedly reduced OVA-induced BAL eosinophils and levels of IL-13 and eotaxin in mice. Since Th2 cells are a major source of IL-13 reduced levels of IL-13 observed after JAK-3 inhibition indicate aberrant functioning of these cells. It was concluded that the reduction in Th2 cells, coupled with inhibition of JAK-3 mediated IL-4 signaling in Th2 like cells leads to a reduction in BAL eosinophils. Although data clearly support the notion that JAK-3 participates in the pathogenesis of pulmonary eosinophilia, interruption of JAK-3 dependent IL-4 signaling may not be the sole cause of the anti-inflammatory effects of JAK-3 inhibition. JAK-3 regulates biological responses of multiple cell types (mast cells, dendritic cells, T cells, macrophages and B cells, Fig. 2) that participate in the pathogenesis of allergy and asthma; it is likely that the observed effect is a consequence of inhibition of JAK-3 in different cell types involved in the disease. In this regard, Verbsky et al. [18] have reported that expression of JAK-3 in nonhematopoietic endothelial cells is also important for the recruitment of Th2 lymphocytes and eosinophils into the lung during OVA-induced inflammation.

Taken together, these findings demonstrate that in the absence of JAK-3, pulmonary inflammation is dampened; moreover JAK-3 appears to be a key regulator of several pathogenic aspects of allergic asthma in mice. However, these studies do not indicate if one or more of these effects is due to the absence of JAK-3 in mast cells alone or its collective absence in other cell types (global JAK-3 deficiency). The specific contribution of mast cell-derived JAK-3 can be evaluated by comparing the responses of mast cell deficient mice

reconstituted with wild-type BMMC or JAK-3-knockout BMMC as described previously [55]. Nevertheless, regardless of its source, JAK-3 inhibition seems to be a novel therapeutic approach as it targets several key mediators involved in allergic inflammation.

Molecular Control of IgE by JAK-3

Since its discovery in 1966 [67], IgE has been linked to the pathogenesis of allergic diseases. In atopic subjects, allergen specific IgE antibodies are synthesized by B cells and secreted into the circulation. IgE antibodies bind to specific receptors (IgE/FcεRI) on the surface of mast cells, basophils, and eosinophils. Once IgE is ligated following exposure to an allergen, these cells are activated releasing a number of pro-inflammatory mediators including histamine, prostaglandins, leukotrienes and various cytokines and chemokines. Thus, IgE synthesis is central to the inflammatory processes in allergic diseases.

Epidemiological studies have demonstrated the significance of IgE in human allergic disorders [68]. Specifically, increased serum IgE levels correlate with disease severity in patients with asthma [68–71]. This has led to the development of a humanized monoclonal antibody, omalizumab, which binds to IgE on the same Fc site as FcεRI [72]. Omalizumab has dual functions; in addition to eliminating free IgE in the blood, it blocks the interaction between IgE and FcεRI receptors, thus preventing mediator release from mast cells, eosinophils and basophils. Moreover, due to decreased plasma IgE levels, expression of IgE receptors is also downregulated in these cells [73, 74]. Clinical studies have demonstrated that omalizumab treatment results in the reduction of eosinophils in the airways of patients with asthma. Notably, the effects of omalizumab are comparable to corticosteroids [75] again demonstrating the importance of IgE in allergic asthma.

The regulatory mechanisms associated with IgE synthesis are complex. Two key events are: (i) isotype switching of B cells to synthesize IgE via IL-4 mediated signaling [76], and (ii) the interaction of CD40 on B cells with CD40 ligand (CD40L) on activated T cells. Evidence suggests that both of these activities are JAK-3 dependent [77, 78]. IL-4 is largely released by Th2 cells and is required for the transcription of germline ϵ in IgM-positive B cells, an important step in IgE class switching [79, 80]. Interestingly, although both IL-4 and closely related IL-13 signal through a classical receptor known as IL-4R, only IL-4 induces JAK-3 phosphorylation [81, 82]. This appears to be due to differences in the structure of the IL-4 receptor. Two types of IL-4 receptors have been identified: i) type I receptor which consists of a IL-4R α subunit, responsible for IL-4 binding and signal transduction, and a common γ chain, and ii) type II receptor which contains IL4R α subunit and a IL-13R α 1 subunit. Since there is no γ chain in type II receptor, JAK-3 only associates with the type I IL-4R [82–84], whereas IL-13 signals via the type II IL-4R. Binding of IL-4 to its cognate receptor on B cells triggers tyrosine phosphorylation of JAK-1 and JAK-3 [85, 86]. It is thought that JAK-1 interacts with the α chain whereas JAK-3 associates with the γ chain of the type I IL-4R. Activated JAK-1 and JAK-3 then phosphorylate STAT-5 and STAT-6 which dimerize and translocate to the nucleus to activate transcription of C ϵ germline [86, 87]. This process is mediated by a GC-associated nuclear protein (GANP) which can modulate JAK-1/JAK-3 to STAT-6 signaling by interaction with the JAK-binding protein, arginine methyltransferase (PRMT) 5 [88]. Like IL-4 deficient mice, STAT-6-knockout

mice produce very low levels of IgE in response to allergen challenge confirming the role of JAK-STAT pathway in B cell class switching [86, 89–91]. Baseline levels of phospho-STAT-1 and phospho-STAT-6 are higher in the blood effector-memory T cells (CD4⁺CD161⁺) of allergic asthmatic patients [92]. The idea that JAK-3 contributes to STAT-6 activation via IL-4 is supported by findings that: i) IL-4 induced STAT-6 DNA binding is not detectable in JAK-3-deficient murine macrophages [93]; ii) a natural flavonoid inhibits IL-4 induced STAT-6 activation only in the cells expressing JAK-3 [94]; and iii) that JAK-3 is constitutively phosphorylated in B cells isolated from the blood of patients with atopic dermatitis (AD) and high serum IgE levels [95]. Additionally, splenic B cells from Nc/Nga mice, which spontaneously develop AD-like skin lesions and exhibit high plasma IgE levels, display increased phosphorylation of JAK-3 in response to CD40L and IL-4, when compared to wild type control mice [95]. Although wild type mice produce high levels of IgE in response to OVA challenge, JAK-3-deficient mice fail to produce any IgE under similar conditions (Malaviya et al., unpublished data). Recently Wilson et al. [29] have demonstrated that a treatment of mice with a JAK-3-specific staurosporine analog inhibits OVA-induced IgE production in wild-type mice. Taken together, these observations implicate JAK-3 in IL-4 mediated STAT-6 activation and overall IgE production by B cells.

The next step in the regulation of IgE switching is mediated by an interaction between B cells and CD4⁺ T cells through ligation of CD40 to its ligand [96, 97]. CD40 is a 48 KDa cell surface receptor that belongs to the TNF-R family [98]. Ligation of B cell CD40 by CD154 (CD40L) expressing T cells stimulates B cell proliferation and differentiation, isotype switching, upregulation of adhesion molecules (e.g. LFA-1), cytokine regulation, the development of germinal center and a humoral memory response [96–102]. Several distinct structural motifs in the CD40 cytoplasmic domain regulate CD40 signaling. These involve both TNF receptor associated factors (TRAFs) and additional signaling proteins, and lead to activation of kinases and transcription factors [97]. A role for CD40-mediated signaling in IgE production is supported by the observation that patients with X-linked hyper-IgM condition have defects in CD40L, and more importantly are unable to undergo isotype switching [103]. It has also been shown that mice with gene disruptions of CD40 or CD40L do not produce IgE in response to antigen challenge *in vitro* or *in vivo* [104, 105].

Although many cytokine receptors including IgE/FcεRI and CD40 lack intrinsic kinase activity, they have the ability to transduce biological signals following ligand binding. For example, studies have shown that CD40 receptor cross linking results in tyrosine phosphorylation of LYN and SYK protein tyrosine kinases [97]. This leads to activation of substrates such as PI3-kinase and PLCγ [97]. Activation of phospholipases induces IP3 generation and calcium mobilization [97]. CD40 ligation also results in activation of serine/threonine kinases including JNK [99]. Since JAK-3 seems to provide a signaling opportunity to receptors that do not possess intrinsic kinase activity, it has been hypothesized that CD40 might utilize the JAK/STAT pathway to generate biological signals. In accord with this hypothesis, experimental evidence indicates that JAK-3 is constitutively associated with the intracellular domain of CD40 in resting B cells and monocytes [96, 101].

Yamada and colleagues [106] have demonstrated a role of JAK-3 in IgE class switch using JAK phosphatase activity of CD45. Specifically, the authors showed that activation of CD45

negatively regulates Ig class switching to IgE in human B cells and that this activity is concomitant with the ability of CD45 to dephosphorylate JAK-1, JAK-3 and STAT-6, but not stress-activated/mitogen activated protein (MAP) kinases. In contrast, Jabara et al. [96] reported that peripheral blood mononuclear cells from patients with JAK-3 deficiency have no defects in IgE synthesis *in vitro*. The authors demonstrated that in the presence of IL-4, B cell stimulation with anti CD40 antibody resulted in similar responses in cells from JAK-3 deficient patients and normal controls with regard to B cell proliferation and IgE isotype switching. Further studies are needed to resolve this discrepancy.

Dendritic Cells and JAK-3

Dendritic cells originate from the bone marrow and are known to be key to innate immune responses [107]. Evidence suggests that they also play a role in adaptive immunity by presenting antigen to naïve T cells and stimulating the maturation of Th2 cells by releasing polarizing cytokines. Although dendritic cells are present throughout the body, their location in the lung is critical for the presentation of inhaled antigen to T cells [108, 109]. A number of studies have shown that dendritic cells accumulate in the lamina propria and the epithelium of the patients with asthma [110–113]. Numbers of dendritic cells also increase with allergen challenge [112, 113]. Studies using animal models of allergic asthma have demonstrated (i) increased numbers of dendritic cells in the airway mucosa of the animals sensitized and challenged with OVA, (ii) naïve mice reconstituted with sensitized dendritic cells develop airway inflammation, and (iii) depletion of dendritic cells impairs airway inflammation. These findings emphasize the important role of dendritic cells in pulmonary inflammation [114, 115].

Dendritic cells present in the naïve lungs are immature and unable to stimulate T cells. Following allergen challenge, dendritic cells migrate to the draining lymph nodes where they present antigen to naïve T cells. When dendritic cells arrive in the lymph nodes, two important events occur; the dendritic cells activate T cells to express CD154, a ligand for CD40, and T cells activate dendritic cells to express CD40 and induce their maturation [116, 117]. Thus CD40/CD40L interaction is an important event in dendritic cell maturation and function. Since CD40/CD40L interaction is a JAK-3 dependent process, one can speculate that JAK-3 also plays a regulatory role in dendritic cell maturation and function. In support of this concept, Revy et al. [101] have shown that JAK-3 is associated with the intracellular domain of CD40 in monocytes, and CD40 mediated activation of monocytes results in JAK-3 activation and phosphorylation. These findings were confirmed by Saemann and colleagues [118] who demonstrated that not only does CD40 ligation induce JAK-3 activation in monocyte-derived dendritic cells, but treatment of these cells with a JAK-3 inhibitor results in retarded CD40 mediated maturation of dendritic cells, defective cytokine production and impaired allostimulatory capacity. Thus it appears that JAK-3 signaling is important for CD40 ligation, dendritic cell maturation, and T cell stimulation, as well as the responses of neighboring cells which may influence these activities. For example, mast cells and dendritic cells co-localize at various sites throughout the body and mast cell-derived mediators regulate the maturation of dendritic cells. This indicates that JAK-3 deficiency in mast cells may also affect the functions of dendritic cells. In this regard, Micheri and colleagues [119] have shown that mast cell derived exosomes induce immature dendritic

cells to up-regulate maturation markers such as CD80, CD86, and CD40 and stimulate antigen presentation capacity. In addition, TNF α which is presynthesized and stored in mast cell granules is released within seconds of mast cell stimulation. TNF α aids in the maturation and migration of dendritic cells from periphery to lymph nodes [120]. Since TNF α release from mast cells is regulated by JAK-3 [121], inhibition of JAK-3 may suppress dendritic cell maturation and antigen presentation after allergen challenge.

Macrophages and JAK-3

Macrophages are among the most long lived cells of the immune system and are recruited to the airways of allergic subjects following allergen challenge [122]. In chronic asthma both monocytes and macrophages are prominent in the airway mucosa and undoubtedly play an important role in disease pathogenesis. Once activated, these cells release cysteinyl leukotrienes, reactive oxygen and nitrogen species, and a variety of cytokines and chemokines, including TNF α , Interferon (IFN) - γ , IL-1, IL-6, CCL2, CCL3, and CXCL8 [123]. These mediators facilitate the recruitment and activation of other inflammatory cells [2]. Nitric oxide is produced by macrophages via an inducible form of the enzyme nitric oxide synthase (iNOS or NOS2), an oxido-reductase that converts arginine to citrulline and nitric oxide [124]. Several different cytokines and inflammatory mediators including IFN- γ , TNF α , IL-1 and bacterial-derived lipopolysaccharide (LPS) induce nitric oxide production by macrophages. Once generated, nitric oxide reacts rapidly with superoxide anion forming peroxynitrite, creating oxidative stress and tissue damage. Elevated levels of iNOS are detected in the lungs of allergen-exposed animals and a treatment with iNOS inhibitor inhibits eosinophilic lung inflammation and allergen-induced bronchial hyperresponsiveness [125]. Although the precise role of JAK-3 in macrophages remains to be determined, findings that JAK-3 mediates IL-6 induced differentiation of macrophages [126] and modulates expression of iNOS following IFN- γ or LPS stimulation [26, 127] suggest a need for further investigation.

Role of JAK-3 in Nonhematopoietic Cell Function in Asthma

Whereas the role of hematopoietic cells in the initiation of inflammatory responses is well established; the contribution of nonhematopoietic cells to allergic inflammation is unclear. Initially, bronchial epithelium was thought to be mere a passive barrier between the external and internal airway environment. However, recent advancement in the biology of airway epithelial cells has led to the conclusion that epithelial cells also contribute to airway inflammation by releasing immunoregulatory cytokines and chemokines [2, 128–130], as well as by interacting with inflammatory cells [4, 131, 132]. It has also been shown that the epithelium is altered in chronic asthma toward a repair phenotype with increased expression of CD44 [5, 133].

The dogma that JAK-3 expression is restricted to hematopoietic cells has been challenged by a number of investigators. For example, Liu and Kern [134] showed that neuregulin-1 induces proliferation of lung epithelial cell lines NCI-H520 and NCI-H441 through activation of JAK-3. Doucet et al [135] also reported that normal fibroblasts express JAK-3 following stimulation with IL-4 and IL-13, whereas myofibroblasts constitutively express

JAK-3. This later study also demonstrated that JAK-3 is activated in myofibroblasts following stimulation with LPS, TNF α or thrombin via CD40 and IL-4R α . Furthermore, using colocalization techniques, Cagnoni and coauthors [98] demonstrated an interplay between JAK-3 and CD40 in cultured human airway epithelial cells supporting a role of JAK-3 in the release of IL-6 and IL-8 by these cells. Taken together, these data suggest that JAK-3 may function as the initial PTK to be activated in allergic inflammation in pulmonary epithelial cells, and that this activation might be key to the proliferation (tissue remodeling) and mediator release by these cells.

Verbsky and colleagues [136] have proposed the idea that expression of JAK-3 in nonhematopoietic cells is required for full expression of inflammatory responses in allergic asthma in mice. Later, using a mouse model of OVA-induced allergic asthma these investigators elegantly showed that JAK-3 mediates the endothelial cell expression of VCAM-1 in airways, as JAK-3-deficient chimeric mice expressed less endothelial-associated VCAM-1 after airway antigen challenge [18]. Since VCAM-1 is required for the recruitment of Th2 cells, macrophages, granulocytes and eosinophils, the authors suggested that JAK-3 activation in airway-associated endothelial cells is essential for eosinophilic airway inflammation.

Concluding Comments

Advances in our understanding of the role of JAK-3 in the pathogenesis of hyperproliferative diseases like cancer and transplant rejection are well documented. Studies conducted in the last decade also implicate JAK-3 in a wide range of immunoinflammatory disorders including type-1 diabetes, allergy, and rheumatoid arthritis suggesting that potent and specific inhibitors of JAK-3 may prove clinically efficacious in numerous diseases. One JAK-3 inhibitor, CP-690550 is in phase I and 2 clinical trials in rheumatoid arthritis, psoriasis, Crohn's disease and renal transplant rejection [137]. Early results have demonstrated efficacy with limited toxicity. In terms of allergic asthma, the molecular mechanisms associated with the pathogenesis of this disease are highly complex; thus the currently available treatments lack the ability to ameliorate the disease symptoms substantially. Accumulating evidence suggests that targeting JAK-3, a potent regulator of multiple pathogenic processes in asthma may be of value.

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Fig. 1. The JAK-STAT signaling pathway

Upon binding ligand, receptor-associated JAKs become activated and mediate phosphorylation of specific receptor tyrosine residues. This leads to the recruitment of specific STATs, which are then tyrosine-phosphorylated. Activated STATs dimerize, translocate to the nucleus, and bind to members of the γ -activated site family of enhancers.

Fig. 2. JAK-3 mediated biochemical events

In both hematopoietic and nonhematopoietic cells JAK-3 mediates diverse biochemical responses important in the pathophysiology of allergic asthma. Inflammatory mediators and cytokines released following JAK-3 activation by one cell may influence the responses of neighboring cell types (shown by dashed arrows). Thus, targeting Jak-3 may permit control of functional activation of multiple cell types important in pathogenesis of allergic disease.

Table I**Inflammatory Mediators and their Possible Roles in Asthma**

Mediators of Asthma	Biological Activity
Histamine	Airway hyper-responsiveness, airway remodeling, vascular permeability, edema
Leukotrienes and prostaglandins	Vascular permeability, airway hyper-responsiveness, vasoconstriction/vasodilation, recruitment of eosinophils, neutrophils, and T cells, expression of adhesion molecules, mucus secretion
TNF α	Recruitment of phagocytic cells, activation of endothelial cells, expression of VCAM-1 and ELAM-1, airway remodeling
Tryptase	Airway remodeling, activate fibrinolysis, recruitment of inflammatory cells, promotes growth and differentiation of fibroblasts, activation of metalloproteinases, airway smooth muscle hypertrophy and hyperplasia, collagen deposition
Chymase	Airway remodeling, neutrophil chemotaxis, vascular permeability changes, mast cell activation, IgE regulation
IL-5	Eosinophil accumulation, maturation and survival
IL-4	Th2 cell differentiation, B cell class switching to IgE and MHC II production
IL-13	Airway hyperreactivity, cellular recruitment and activation
Chemokines (IL-8, MCP-1 etc.)	Recruitment and activation of inflammatory cells and pathogenesis of airway remodeling