Kinases as Novel Therapeutic Targets in Asthma and Chronic Obstructive Pulmonary Disease

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Abstract—Multiple kinases play a critical role in orchestrating the chronic inflammation and structural changes in the respiratory tract of patients with asthma and chronic obstructive pulmonary disease (COPD). Kinases activate signaling pathways that lead to contraction of airway smooth muscle and release of inflammatory mediators (such as cytokines, chemokines, growth factors) as well as cell migration, activation, and proliferation. For this reason there has been great interest in the development of kinase inhibitors as anti-inflammatory therapies, particular where corticosteroids are less effective, as in severe asthma and COPD. However, it has proven difficult to develop selective kinase inhibitors that are both effective and safe after oral administration and this has led to a search for inhaled kinase inhibitors, which would reduce systemic exposure. Although many kinases have been implicated in inflammation and remodeling of airway disease, very few classes of drug have reached the stage of clinical studies in these diseases. The most promising drugs are p38 MAP kinases, isoenzyme-selective PTK kinases, Janus-activated kinases, and Syk-kinases, and inhaled formulations of these drugs are now in development. There has also been interest in developing inhibitors that block more than one kinase, because these drugs may be more effective and with less risk of losing efficacy with time. No kinase inhibitors are yet on the market for the treatment of airway diseases, but as kinase inhibitors are improved from other therapeutic areas there is hope that these drugs may eventually prove useful in treating refractory asthma and COPD.

I. Introduction

Asthma and chronic obstructive pulmonary disease (COPD) both cause obstruction of the airways and involve chronic inflammation of the respiratory tract. Both are very prevalent diseases and are increasing throughout the world.

Asthma is now the most common chronic disease in developed countries, affecting up to 20% of the population, and is increasing, particularly in developing countries (Beasley et al., 2000). It still causes death and leads to hospital admissions because of acute exacerbations. Although asthma is usually well controlled with inhaled corticosteroids, many patients with asthma are poorly controlled in the real world as adherence to this treatment is poor and inhaler use is often inadequate (Partridge et al., 2006; Braido et al., 2013). Approximately 20% of asthma patients are particularly difficult to manage, and 3–5% of patients have severe disease that is not controlled even by maximal inhaled treatment with an inhaled corticosteroid and a long-acting β2-agonist (Chung and Wenzel, 2007).

ABBREVIATIONS: AHR, airway hyperresponsiveness; AMPK, adenosine monophosphate kinase; AP-1, activator protein-1; ASK, apoptosis signal-regulatory kinase; ASM, airway smooth muscle; ATP, adenosine triphosphate; AZD2014, 3-(2,4-bis((S)-3-methylmorpholino)pyrido[2,3-d]pyrimidine-7-yl)-N-methylbenzamide; Bay 65-1942, (TE)-7-[2-(cyclopentylmethoxy)-6-oxo cyclohexa-2,4-dien-1-ylidene]-5-[[3S]-piperidin-3-yl]-4,8-dihydro-1H-pyrido[2,3-d][1,3]oxazin-2-one; BTK, Bruton’s tyrosine kinase; COPD, chronic obstructive pulmonary disease; CT99021, 6-[[2-[(4-Dichlorophenyl)-5-(5-methyl-1H-imidazol-2-yl)-2-pyrimidinyl]amino]ethyl]amino]-3-pyridinedicarbonitrile; CXCL8, interleukin-8; EGFR, epidermal growth factor receptor; ERK, extracellular regulating kinase; FEV1, forced expiratory volume in 1 second; FGF(R), fibroblast growth factor (receptor); GM-CSF, granulocyte-macrophage colony stimulating factor; GR, glucocorticoid receptor; GSK2296957, 2-(6-((1H-indol-4-yl)1H-indazol-4-yl)-5-((4-isopropylpiperazin-1-yl)methyl)oxazole hydrochloride; GSK3β, glycogen synthase-3β; Hck, hematopoietic cell kinase; HDAC2, histone deacetylase-2; hsp, heat shock protein; IFN, interferon; IgE, immunoglobulin E; IKK, inhibitor of kB kinase; IL, interleukin; IRAK, interleukin-1 receptor-associated kinase; ITAM, immunoreceptor-tyrosine-based activation motif; JAK, Janus-activated kinase; JNK, Jun NH2-terminal kinase; LAS 189386, 1-[[2-[[1(S),4S)-2,5-diazabicyclo[2.2.1]hept-2-yl]pyridin-4-yl]-N-pyrazin-2-yl]amino]-1H-indazol-3-amine; LPS, lipopolysaccharide; LY294002, 2-Morpholin-4-yl-8-phenyl chromen-4-one; MAPK, mitogen-activated protein kinase; MAPKAPK or MK2, MAP-activated protein kinase; mTOR, mammalian target of rapamycin; MKP, MAP kinase phosphatase; MPP, mammalian target of rapamycin; NF-κB, nuclear factor-kappa B; NGF, nerve growth factor; PKC, protein kinase C; PTEN, phosphatase and tensin homolog deleted from chromosome 10; ROCK, Rho-associated protein kinase; ROI, reactive oxygen intermediate; Rho-kinase; RTK, receptor tyrosine kinase; SD-282, indole-5-carboxamide; SHIP, Src homology 2 domain-containing inositol 5-phosphatase; STAT, signal transducer of activators of transcription; Syk, spleen tyrosine kinase; TAK, transforming growth factor-β-activated kinase; TGFB, transforming growth factor; TLR, Toll-like receptors; TNF, tumor necrosis factor; VEGF(R), vascular endothelial growth factor (receptor).
2014; Hekking et al., 2015). There is a need to develop more effective therapies for these patients who consume a disproportionate amount of medical resources.

COPD is also very prevalent, affecting about 10% of people over 45 years in developed countries and increasing especially in developing countries (Barnes et al., 2015). The increasing world-wide prevalence of COPD reflects aging of the population because it is a disease of elderly people. COPD has a high morbidity and is one of the leading causes of hospitalization and loss of time from work. It has a rising mortality and is now the third most common cause of death in developed countries (Lozano et al., 2012). No current treatments, including corticosteroids, reduce disease progression or mortality and have relatively little effect (~20% reduction) in preventing exacerbations, reflecting their lack of anti-inflammatory effects in this disease. There is therefore an enormous unmet need for the development of new treatments for COPD that target the underlying inflammatory process and aberrant repair mechanisms (Barnes, 2013b).

Although asthma and COPD are distinct inflammatory diseases, some patients with COPD may have features of asthma such as eosinophilic inflammation and greater airway reversibility, whereas some patients with asthma may have features of COPD, such as neutrophilic inflammation, reduced reversibility, and reduced responsiveness to corticosteroids. These patients have been described as having asthma-COPD overlap syndrome, although several phenotypes are included in this label (Bateman et al., 2015; Postma and Rabe, 2015; Barnes, 2016). Improved treatment of patients with asthma-COPD overlap is needed (Barnes, 2015b).

Thus, asthma and COPD are very common chronic inflammatory diseases of the respiratory tract with several unmet needs for more effective medication. Because chronic inflammation involves the activation of multiple kinase signaling pathways (Cohen, 2002), this has provided a rationale for the development of selective kinase inhibitors for the treatment of asthma and COPD, particularly in patients with severe disease that cannot be controlled by existing therapies. In view of the role of protein kinases in chronic inflammation and tissue remodeling, the use of kinase inhibitors is a logical approach. Furthermore, there is increasing evidence that kinases are also involved in corticosteroid resistance, which is a feature of severe asthma and COPD (Barnes and Adcock, 2009). Development of kinase inhibitors for inflammatory diseases has proven to be problematic because of the adverse effects when these drugs are given systemically. Theoretically, this could be overcome by the development of inhaled inhibitors, but it has proven difficult to develop kinase inhibitors that have local activity in the airways. This review summarizes the current evidence for the effects of protein kinase inhibitors in inflammatory airway diseases.

II. Role of Kinases in Asthma and COPD

Both asthma and COPD are characterized by a chronic inflammatory response in the respiratory tract that involves the recruitment and activation of several inflammatory and immune cells and the secretion of multiple inflammatory mediators, including lipid mediators, cytokines, chemokines, and growth factors, and well as enzymes that generate inflammatory products or are involved in tissue remodeling (Barnes, 2008a,b). Many of these inflammatory proteins are regulated by proinflammatory transcription factors, such as nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) that are regulated by kinase signaling pathways. Furthermore, these mediators act on surface receptors on target cells in the airways that also activate kinase pathways. In the case of asthma, allergen may be an important driver of the allergic inflammatory response and this involves the crosslinking of allergens with immunoglobulin E (IgE) bound to mast cells, with the activation of kinase s that result in release of preformed mediators, such as histamine and the synthesis and release of lipid mediators, such as cysteinyl-leukotrienes and prostaglandins (Barnes, 2011; Galli and Tsai, 2012). Allergen is also taken up by dendritic cells and this activates kinase pathways, leading to the recruitment of T-helper 2 (Th2) lymphocytes to the airways that drive eosinophilic inflammation and airway hyperresponsiveness, which are the defining features of asthma (Barnes, 2011). Innate immunity also plays a key role in asthma, particularly severe and nonallergic asthma that is driven in innate lymphoid-2 cells (Scanlon and McKenzie, 2012). Innate immune mechanisms and the activation of pattern recognition receptors, such as Toll-like receptors (TLR) that are activated by cigarette smoke and wood smoke (which are key risk factors for the development of COPD), and by pathogens, such as colonizing bacteria. Mitogen-activated protein kinases (MAPK) play a key role in the activation of both innate and adaptive immune cells, resulting in the secretion of multiple cytokines, chemokines, and growth factors that then activate further waves of inflammation (Arthur and Ley, 2013). COPD represents an acceleration of the normal aging process in lungs and these pathways of cellular senescence are also regulated by multiple interacting kinase pathways (Mercado et al., 2015).

III. Kinases

Kinases are enzymes that transfer the γ-phosphate of adenosine-triphosphate (ATP) to hydroxyl-bearing substrates and thus phosphorylate proteins, lipids, and sugars, which results in activation of enzymes, cellular translocation, or interaction with other proteins and play a critical role in signal transduction. Over 500 kinase genes have now been recognized in humans,
making up ~2% of the genome (the “kinome”), and over 30% of proteins are regulated by phosphorylation (Manning et al., 2002). These are organized into distinct families, such as mitogen-activated protein kinases (MAPK). The majority of kinases phosphorylate serine (Ser) or threonine (Thr) residues (serine/threonine kinases); some act on tyrosine (Tyr) residues (tyrosine kinases), whereas other act on both types (dual-specificity kinases). Tyrosine-kinases may act as receptors for ligands such as growth factors, such as epidermal growth factor receptor (EGFR). Because kinases play such a critical role in signaling, they are carefully regulated by phosphatases, which remove phosphate groups and thus reverse the effects of a specific kinase. Kinases are comprised of several domains, including a well conserved catalytic domain that binds ATP. Although the majority of kinases target proteins, some target lipids (such as phosphatidylinositol and sphingosine), carbohydrates (such as hexokinase), or nucleotides, such as thymidine kinase.

Protein kinases are selective for Ser/Thr or Tyr residues in their target proteins and recognize a consensus sequence around these residues rather than all of the residues on a particular target protein (Fabbro et al., 2015). However, a particular kinase is usually not specific for a particular protein but may act on several proteins sharing this consensus sequence. Pseudosubstrates bind to the kinase but lack the amino acid that is phosphorylated, so that they effectively act as an inhibitor of the kinase. Kinases are organized into signaling pathways, whereby one kinase phosphorylates a downstream kinase, which then phosphorylates the next downstream kinase in the sequence. Each kinase may phosphorylate different kinases so that several signaling cascades may be activated and there may be crosstalk between different kinase signaling pathways, resulting in amplification or inhibition of signaling. This makes signaling pathways highly complex, and it is often difficult to predict the effects of inhibiting a single kinase. Furthermore, there are complex feedback inhibitory loops that may result in unexpected effects of interrupting kinase pathways.

A. Kinase Inhibitors

The recognition that kinases are key regulators of cell proliferation, apoptosis, and inflammation and that mutation of kinase genes is an important mechanism in many immune and inflammatory diseases has prompted a search for selective small molecule inhibitors in many human diseases (Cohen, 2002). Many small molecule kinase inhibitors have now been approved for use in cancer (Wu et al., 2015c), and although most of the drug discovery efforts have focused on malignancy, kinase inhibitors are now seen as important potential therapies for many chronic inflammatory and immune disorders (Cohen, 2014). Indeed, kinase inhibitors are now the second largest group of drugs in development after G-protein-coupled receptor ligands and account for ~30% of drug discovery projects in major pharmaceutical companies (Wu et al., 2015c). Because both asthma and COPD involve inflammatory and immune mechanisms, there have been great efforts to develop kinase inhibitors as new therapies, particularly targeting areas of unmet need (Adcock et al., 2006).

Development of specific kinase inhibitors has proven to be very difficult because the catalytic site is well conserved between different kinases and most inhibitors target the ATP-binding site. This means that many kinase inhibitors are poorly selective and often have several off-target effects on structurally related kinases, which may increase the risks of adverse effects. A further problem with kinase inhibitors in cancer therapy is the development of resistance due to site mutations at the ATP-binding pocket. With chronic treatment of inflammatory diseases there may also be a gradual wearing off of efficacy of kinase inhibitors due to the upregulation of compensatory pathways. For example, p38 MAPK inhibitors for rheumatoid arthritis may lose efficacy because of upregulation of other proinflammatory kinase pathways (Firestein et al., 2008). This might be less of a problem if more upstream kinases are targeted, although the risks of side effects may be even greater.

Most kinase inhibitors so far have been given orally, so that systemic side effects are common and often dose-limiting. As a means of reducing adverse effects, there has been great interest in the development of allosteric inhibitors that target less conserved sites in the kinase domain or remote sites (Wu et al., 2015b). These drugs may not need to be of such high potency that is needed to compete with the high concentrations of ATP at the catalytic site and may also be more resistant to mutations. Allosteric kinase inhibitors, such as trametinib, are now in clinical development, at least for the treatment of cancer. For example, allosteric inhibitors that bind to a distal site have now been discovered for p38 MAPK (Comess et al., 2011). Inhaled delivery may also reduce the risk of adverse effects by targeting the drug to the airways. So far it has proven difficult to develop inhaled kinase inhibitors that are effective because they do not have topical activity. This is related to the fact that kinases are intracellular targets, so the drug properties that target cell penetration also increase oral bioavailability and rapid transport across the lung into the systemic circulation.

IV. Nuclear Factor-κB inhibition

A. Role in Asthma and Chronic Obstructive Pulmonary Disease.

The proinflammatory transcription factor NF-κB is activated in asthma and COPD and switches on multiple inflammatory genes, including cytokines, chemokines, proteases, and inhibitors of apoptosis, resulting
in amplification of the inflammatory response (Barnes and Karin, 1997; Hart et al., 1998; Di Stefano et al., 2002; Caramori et al., 2003). NF-κB activation, measured by nuclear localization of p65 (RelA), is seen predominantly in airway epithelial cells and macrophages and may be activated by inflammatory cytokines (such as TNF-α and IL-1β), oxidative stress, allergens, and bacteria. NF-κB may play an important amplifying role in acute exacerbations of asthma and COPD, which are commonly triggered by upper respiratory tract viruses, such as rhinovirus (Caramori et al., 2003). NF-κB is activated by rhinovirus in airway epithelial cells (Footitt et al., 2016; Papi et al., 2013) and there is marked activation of NF-κB in the lungs of patients who died from asthma (Caramori et al., 2009).

The molecular pathways involved in NF-κB activation include several kinases, which are targets for inhibition. The classic (canonical) pathway for inflammatory stimuli and infections to activate NF-κB signaling involve the IKK (inhibitor of κB kinase) complex, which is composed of two catalytic subunits, IKK-α and IKK-β, and a regulatory subunit, IKK-γ (or NF-κB essential modulator) (Hayden and Ghosh, 2012). The IKK complex phosphorylates NF-κB-bound IκBs, targeting them for degradation by the proteasome and thereby releasing NF-κB dimers that are composed of p65 and p50 subunits, which translocate to the nucleus where they bind to κB recognition sites in the promoter regions of inflammatory and immune genes, resulting in their transcriptional activation (Fig. 1). This response depends mainly on the catalytic subunit IKK-β (also known as IKK2), which carries out IκB phosphorylation. Although the canonical pathway has been studied most extensively in asthma and COPD, there is a noncanonical (alternative) pathway, which involves the upstream kinase NF-κB-inducing kinase (NIK) that phosphorylates IKK-α homodimers and releases RelB and processes p100 to p52 in response to certain members of the TNF family, such as lymphotoxin-β (Sun, 2012). This pathway switches on different gene sets and may mediate different immune functions from the canonical pathway. Dominant-negative IKK-β inhibits most of the proinflammatory functions of NF-κB, whereas inhibiting IKK-α has a role only in response to limited stimuli and in certain cells such as B-lymphocytes. The noncanonical pathway is involved in development of the immune system and in adaptive immune responses, but its role in asthma and COPD has not been explored in detail. The coactivator molecule CD40 is expressed on antigen-presenting cells, such as dendritic cells and macrophages, activates the noncanonical pathway when it interacts with CD40L expressed on lymphocytes, and may amplify allergic responses in asthma (Lombardi et al., 2010).

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**Fig. 1.** Nuclear factor-κB signaling pathway. The canonical (classic) pathway is activated by inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin(IL)-1β, and lipopolysaccharide (LPS), leading to phosphorylation of inhibitor of κB (IκB), which is ubiquitinated and degraded by the proteasome releasing p65 and p50 to translocate to the nucleus, where they bind to κB DNA recognition sequence, leading to the activation of cytokines, chemokines, and proteases. The noncanonical pathway is activated by CD40 and lymphotoxin(LT)-β, which activate NF-κB-inducing kinase (NIK), resulting in activation IKKα homodimers, which phosphorylate RelB/p100, generating RelB/p50 complexes that translocate to the nucleus and switches on immune genes. IKK-β may be inhibited by several compounds (shown in the green box), but no inhibitors of the non-canonical pathway have been identified.
stimulation induces emphysema in mice, and soluble CD40L is increased in plasma of COPD patients and correlates with the degree of emphysema, suggesting a possible pathogenic role (Shigeta et al., 2012). Indirect evidence for the involvement of the noncanonical pathway is the activation of NIK and phosphorylation of IKK-α in airway epithelial cells after exposure to cigarette smoke and oxidative stress (Chung et al., 2011). On the other hand, RelB overexpression in mice inhibits cigarette smoke neutrophilic inflammation through stimulation of cyclooxygenase-2 (McMillan et al., 2011).

B. Inhibitors.

Inhibition of NF-κB is a logical approach to treating inflammation in asthma and COPD because there is evidence for its activation and increased activation of multiple proinflammatory genes. However, deletion of NF-κB related genes in mice is lethal or animals develop severe septicemia, indicating that there are risks of NF-κB inhibition (Hayden and Ghosh, 2012). Corticosteroids inhibit NF-κB activation in patients with asthma (Hart et al., 2000), but there is resistance to this effect in patients with severe asthma and COPD (Barnes and Adcock, 2009). Theophylline is also reported to inhibit NF-κB in human airway epithelial cells through IKK inhibition, although high concentrations are needed for this effect (Ichiyama et al., 2001). Several naturally occurring compounds, such as plant polyphenols, have also been reported to have inhibitory effects on NF-κB. For example, resveratrol inhibits NF-κB and inflammatory cytokine release from COPD epithelial cells in vitro (Donnelly et al., 2004). The green tea extract epigallocatechin-3-gallate inhibits IKK activity (IKK-α as well as IKK-β) in epithelial cells in vitro (Yang et al., 2001).

Several IKK-β inhibitors of differing chemical classes have been developed and in vitro have demonstrated inhibition of p65 nuclear translocation and the activation of inflammatory genes after various stimuli as well as anti-inflammatory effects in vivo in animal models (Suzuki et al., 2011). Both direct and allosteric inhibitors have been developed. A decoy oligodeoxyribonucleotide against NF-κB markedly inhibits allergic lung inflammation in an ovalbumin-sensitized mice with marked inhibition of Th2 cytokine release (Desmet et al., 2004). A selective IKK-β inhibitor IMD-0354 [N-[3,5-bis(trifluoromethyl)phenyl]-5-chloro-2-hydroxybenzamide] also reduced allergic inflammation and airway hyperresponsiveness in this murine model, predominantly through inhibition of T cell trafficking (Sugita et al., 2009; Maslanka et al., 2016). Another IKK-β inhibitor [Bay 65-1942 ((7E)-7-[2-cyclopropylmethoxy]-6-oxocyclohexa-2,4-dien-1-ylidene]-5-[(3S)-piperidin-3-yl]-4,8-dihydro-1H-pyrido[2,3-d][1,3]oxazin-2-one] is also effective at inhibiting pulmonary allergic inflammation in a rat model (Ziegelbauer et al., 2005). Yet another IKK-β inhibitor TPCA-1 is effective in a mouse model of neutrophilic inflammation induced by inhaled endotoxin, although it was not effective in a neutrophil elastase-induced inflammation model (Birrell et al., 2006). IKK-β inhibitors have also been ineffective in cigarette smoke-induced lung inflammation in mice (Rastrick et al., 2013). Several IKK-β inhibitors suppressed the release of cytokines and chemokines from human airway smooth muscle and epithelial cells and were mimicked by adenovirus mediated delivery of a dominant-negative IKK-β inhibitor (Catley et al., 2006; Newton et al., 2007). To avoid potential systemic exposure, an inhaled IKK-β inhibitor (PF-104) was developed to inhibit neutrophilic lung inflammation induced by inhaled endotoxin in mice (Somers et al., 2009). Ainsliadimer-A is found in a Chinese herbal product that has been described as a potent and selective inhibitor of IKKα/β, thus inhibiting the classic and alternative pathways of NF-κB (Dong et al., 2015). It inhibits the release of inflammatory cytokines after LPS injection in mice, so has potential as an anti-inflammatory drug.

Because NF-κB plays a critical role in host defense against pathogens, there are concerns that inhibitors may increase the risk of infection. There is also an uncertain relationship to cancer, because NF-κB may drive tumorigenesis, but its inhibition may also increase cell proliferation. Limited numbers of clinical trials have been conducted. IMD-1041, a prodrug of the IKK-β inhibitor IMD-0354, was tested in patients with COPD for anti-inflammatory effects, but the trial was terminated because of side effects of the medication (ClinicalTrials.gov: NCT00883584). Other IKK-β inhibitors are in clinical trials for other inflammatory diseases, but no results have been published and no products have been approved. It is likely that an inhaled formulation will be necessary to reduce adverse effects.

V. Mitogen-Activated Protein Kinase Inhibition

MAPK are a large family of highly conserved serine/threonine protein kinases that are involved in signal transduction (Johnson and Lapadat, 2002). Although originally shown to be activated by mitogens, they are activated by many stimuli, including cytokines, growth factors, and various stresses (osmotic, oxidative, heat shock) and result in diverse cellular responses, such as proliferation, differentiation, mitosis, cell survival, apoptosis, and inflammatory gene expression. MAPK are normally inactive in the resting cell and become activated by phosphorylation, usually of a threonine and a tyrosine residue. Several scaffold proteins are associated with MAPK pathways and appear to direct signaling within the cell by directing proteins to particular cellular compartments and may also modulate signaling. Typically MAPKs operate in a three-tiered cascade regulated by successive phosphorylation starting with
activation of MAP kinase kinase kinases (MAP3K), then MAP kinase kinases (MAP2K), and finally MAPK (Fig. 2). The major MAPK pathways involved in inflammatory diseases are ERK (extracellular regulating kinase), p38 MAPK, and JNK (c-Jun NH2-terminal kinase). Upstream kinases include TGFβ-activated kinase-1 (TAK1) and apoptosis signal-regulating kinase-1 (ASK1). Downstream of p38 MAPK is MAPK activated protein kinase 2 (MAPKAPK2 or MK2). Several classes of inhibitor are now developed and shown in green boxes.

A. p38 Mitogen-Activated Protein Kinase

The p38 MAPK family comprises α-, β-, γ-, and δ-isoforms, which are highly conserved proline-directed protein kinases that are activated by several extracellular stresses, including oxidative and osmotic stress and inflammatory cytokines. P38 MAPK is activated by phosphorylation of Thr180 and Tyr182 in the Thr-Gly-Tyr motif of the activation loop, which allows p38 to bind ATP at its catalytic binding site to transfer the γ-phosphate group from ATP to the protein targeted for phosphorylation. P38 MAPK is activated by upstream kinases MKK3, MKK4, and MKK6, which are themselves activated by MAP3Ks, such as ASK1 (apoptosis signal-regulatory kinase 1), TAK1 (transforming growth factor-β-activated kinase-1), MLK3 (mixed-lineage kinase 3), and MAP kinase kinase kinase (MEKK3), which are activated by many stimuli, including inflammatory cytokines, growth factors, and oxidative stress. P38 MAPK targets many proteins, including other kinases, transcription factors, and cytoskeletal proteins, resulting in multiple inflammatory effects. P38 MAPK activates over 60 downstream targets, so may be a key point for inhibition (Trempolec et al., 2013). P38α and p38β isoforms are widely distributed and target a downstream target MAPK activated protein kinase 2 (MAPKAPK2 or MK2), which mediates many of these effects. P38α, via activation of MK2, increases inflammation by stabilizing mRNA transcripts of some cytokines, such as TNFα, by phosphorylating adenosine-uracil-binding proteins, such as tristeraprolin, that bind to the adenosine-uracil-rich 3′-untranslated regions of mRNAs to regulate their stability (Dean et al., 2004; Brook et al., 2006). MK2 also phosphorylates heat shock protein(hsp)-27, which has been used as a biomarker of p38α activation. P38α potentiates the proinflammatory effects of NF-κB by phosphorylation of histone H3 at the promoter sites on proinflammatory cytokine and chemokine genes to increase binding of NF-κB binding to their promoter regions (Saccani et al., 2002). P38γ and p38δ isoforms have a more restricted distribution, particularly macrophages, and target mainly transcription factors.

1. Role in Asthma and Chronic Obstructive Pulmonary Disease. There is considerable evidence that p38
MAPK is activated in asthma and COPD and plays an important role in driving chronic inflammation as well as corticosteroid insensitivity (Chung, 2011). P38α is activated in airway epithelial cells and macrophages by lipopolysaccharide (LPS) and cigarette smoke and mediates the release of inflammatory proteins, such as CXCL8 (IL-8), IL-6, TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Selective inhibitors of p38α inhibit the release of these cytokines from human macrophages activated by cigarette smoke and LPS (Koch et al., 2004; Smith et al., 2006). However, p38α inhibitors did not cause maximal inhibition in macrophages and it is possible that other isoforms, such as p38β are important in macrophages (Smith et al., 2006). Alveolar macrophages from patients with severe asthma show increased activation of p38, which may reflect a reduction in the endogenous inhibitor of p38 MAPK phosphatase-1 (MKP1, also known as dual specificity phosphatase-1) (Bhavsar et al., 2008). In mice exposed to cigarette smoke and to ozone, a p38α inhibitor SD-282 significantly suppressed the neutrophil inflammatory response, whereas corticosteroids were ineffective (Medicherla et al., 2007; Williams et al., 2008). This suggests that p38α may play an important role in pulmonary neutrophilic inflammation in COPD. Indeed, phosphorylated p38α shows increased expression in alveolar macrophages, epithelial cells, and CD4+ and CD8+ lymphocytes in lungs from COPD patients (Renda et al., 2008; Gaffey et al., 2013).

P38α is also an important mediator of inflammation in response to upper respiratory tract virus infections and so may play a role in acute exacerbations of asthma and COPD. Rhinovirus-induced release of chemokines from alveolar macrophages is associated with activation of p38α (Hall et al., 2005) and a p38α inhibitor suppresses release of inflammatory cytokines from human airway epithelial cells infected with rhinovirus (Griego et al., 2000). H. influenzae, bacteria commonly colonizing the lower airways and causing acute exacerbations of COPD patients, also activates p38 in airway epithelia cells and mediates the release of CXCL8 (Wang et al., 2003). H. influenzae also stimulates the secretion of the mucin MUC5AC from epithelial cells via p38α activation, suggesting that inhibition of this pathway may reduce mucus hypersecretion in COPD patients (Shen et al., 2008).

P38 activation is also important in mediating allergic inflammation. An inhaled p38α antisense oligonucleotide was effective in inhibiting eosinophilic inflammation and airway hyperresponsiveness after allergen challenge in an ovalbumin-sensitized mouse model (Duan et al., 2005b). A selective p38α inhibitor, indole-5-carboxamide (SD-282), similarly reduced allergic inflammation, airway hyperresponsiveness and airway remodeling in a chronic allergen exposure mouse model (Nath et al., 2006). P38α may play an important role in allergic inflammation through the activation of the transcription factor GATA3, which regulates the Th2 cytokines IL-4, IL-5, and IL-13, because p38 phosphorylates GATA3 in the cytoplasm of Th2 cells so that it is able to translocate to the nucleus to activate Th2 cytokine genes (Maneechotesuwan et al., 2007). A p38 inhibitor, by preventing GATA3 phosphorylation, inhibits the expression of Th2 cytokines (Maneechotesuwan et al., 2009). P38α also prolongs eosinophil survival by enhancing the effects of IL-5 and a p38 inhibitor promotes apoptosis (Kankaanranta et al., 1999).

P38α appears to play an important role in the corticosteroid insensitivity that occurs in severe asthma and COPD (Barnes, 2013a). The corticosteroid insensitivity induced by IL-2 and IL-4 or by IL-13 in peripheral blood mononuclear cells (PBMC) is reversed by a p38α inhibitor (Matthews et al., 1999; Irusen et al., 2002). Corticosteroids increase the expression of MKP-1 and thus inhibit p38 activation. In mice with deletion of MKP-1 corticosteroid lose their anti-inflammatory effects (Abraham et al., 2006). In addition, IL-1β result in phosphorylation of the glucocorticoid receptor (GRα) at Ser211 via p38α activation, preventing translocation to the nucleus (Szatmary et al., 2004). P38α is activated in PBMC from patients with severe and steroid-resistant asthma and is correlated with GRα phosphorylation and reduced responsiveness to corticosteroids (Mercado et al., 2012; Li et al., 2015). Furthermore, a p38α inhibitor is able to restore corticosteroid responsiveness in these cells. Similarly, in PBMC from COPD patients the reduced responsiveness to corticosteroids was reversed by treatment with a p38α inhibitor (Khorasani et al., 2015). P38γ also phosphorylates GRα and reduces nuclear translocation, resulting in corticosteroid insensitivity (Mercado et al., 2011b). P38γ is increased in PBMC of patients with severe asthma and may be reduced by treatment with the long-acting β2-agonist formoterol, which thereby reduces corticosteroid resistance in these cells (Mercado et al., 2011b).

B. p38 Inhibitors

There is a strong rationale for the development of p38 inhibitors to reduce chronic inflammation (both neutrophilic and eosinophilic), to reduce corticosteroid resistance, and to reduce structural changes, particularly in patients with severe asthma and COPD. Selective p38α inhibitors have been rather effective in asthmatic and COPD cells in vitro and in vivo animal models of these diseases. Several p38 inhibitors have been developed for clinical use, and these drugs are mainly selective for p38α and p38β isoforms (Norman, 2015). These drugs have been studied largely in patients with rheumatoid arthritis, but have been disappointing because they have been dose-limited by side effects and have lost efficacy after repeated administration due to the development of compensatory signaling pathways. Most of the clinical studies have been in COPD, where there is a great need to
develop effective anti-inflammatory treatments because corticosteroids are largely ineffective, and eight p38α inhibitors are known to have entered clinical trials in COPD (Norman, 2015). An oral p38α inhibitor dilmapimod (SB-681323) significantly reduced phosphorylated hsp-27 (demonstrating target engagement) and TNF-α release from circulating leukocytes of COPD patients, demonstrating proof of mechanism (Singh et al., 2010). Losmapimod is an oral dual p38α/β inhibitor that had no effect on sputum neutrophils in a phase II study of about 300 COPD patients over 12 weeks but reduced phosphorylation of hsp-27 and reduced plasma fibrinogen by approximately 11% (Lomas et al., 2012). In the largest clinical trial of about 600 COPD patients studied over 24 weeks, three doses of losmapimod were ineffective on exercise tolerance (6 minute walking distance), although there was a trend for reduction in exacerbations; however, there were side effects at the highest dose, particularly dermatological effects (Watz et al., 2014).

In a post hoc analysis in patients with low blood eosinophils (<2%), there was a dose-related reduction in exacerbations with a 55% reduction at the highest dose and a small improvement in FEV₁ (15 mg)(Marks-Konczalik et al., 2015). PH-797804 [(aS)-3-[3-bromo-4-[[2,4-difluorobenzyl]oxy]-6-methyl-2-oxopyridin-1(2H)-yl]-y1-N, 4-dimethylbenzamdePF-03715455=1-[5-tertbutyl-2-(3-chloro-4-hydroxyphenyl)pyrazol-3-yl]-3-[[2-[3-[2-(2-hydroxyethylsulfanyl)phenyl]-1,2,4]triazolo[4,3-a]pyridin-6-yl]sulfanyl]phenyl[methyl]urea] is an oral p38α-selective inhibitor, which increased trough FEV₁ values and dyspnea at some doses in a 6-week study of 230 patients with COPD (MacNee et al., 2013). Because of the problems of side effects at high oral doses of p38 inhibitors, several inhaled drugs have been developed, but no results in airway disease have been reported (Millan et al., 2011). An inhaled p38 inhibitor, PF-03715455 [1-[5-tertbutyl-2-(3-chloro-4-hydroxyphenyl)pyrazol-3-yl]-3-[2-[3-[2-(2-hydroxyethylsulfanyl)phenyl]-1,2,4]triazolo[4,3-a]pyridin-6-yl]sulfanyl]phenyl[methyl]urea], had no significant effect on sputum inflammatory biomarkers although an oral p38 inhibitor had some suppressive effect in the same population (Singh et al., 2015). RV-568 is a potent inhaled p38 inhibitor that inhibits all four isoforms of p38α and also the Src family kinase Hck and is referred to as a narrow spectrum kinase inhibitor, which significantly increased FEV₁ and reduced sputum malondealdehyde after 2 weeks of administration (Russell et al., 2013). No clinical studies of p38 inhibitors in asthma have been reported, and most of the drugs in development for COPD have now been discontinued.

C. Jun NH2-terminal Kinase Inhibitors

JNK is an important activator of the proinflammatory transcription factor activator protein-1 (AP-1), which regulates many inflammatory genes in asthma and COPD. Sensitized mice exposed to inhaled allergen show increased activation of JNK in airway epithelial cells and lung. Furthermore, a JNK inhibitor SP600125 (1,9-pyrazoloanthrone) suppressed the inflammatory response to allergen and reduced mucus hypersecretion and airway hyperresponsiveness, suggesting a role for JNK in allergic inflammation (Nath et al., 2005; Wu et al., 2015a). TNF-α prolongs the survival of human eosinophils via activation of JNK, which thereby sustains eosinophilic inflammation (Kankaanranta et al., 2014). JNK may also play a role in airway remodeling, because SP600125 also reduces airway smooth muscle proliferation after chronic inhaled allergen exposure in mice (Eynott et al., 2003). In JNK-deficient mice, the fibrosis after chronic exposure to allergen (house dust mite) was significantly reduced (van der Velden et al., 2014). JNK regulates airway smooth muscle proliferation and increased collagen synthesis through the activation of transforming growth factor (TGF)-β (Xie et al., 2007) and this is mediated through the activation of AP-1 (Lee et al., 2006b). JNK also plays a role in CD40-mediated class switching in B cells, and SP600125 inhibits the formation of IgE transcripts in B cells (Jabar and Geha, 2005). JNK activation is also involved in corticosteroid resistance in severe asthma patient, because AP-1 competes with GRα and there is evidence for JNK activation in PBMC of patients with steroid-resistant asthma (Adcock et al., 1995; Lane et al., 1998). The corticosteroid resistance induced by IL-2 and IL-4 in PBMC is mediated by activation of JNK, resulting in phosphorylation of Ser226 in GRα to prevent its nuclear localization (Kobayashi et al., 2012). JNK activation is also involved in the corticosteroid resistance induced by rhinovirus infection of airway epithelial cells, which is reduced by a JNK inhibitor and completely reversed by a combination of JNK and IKK-β inhibitors (Papi et al., 2013). JNK activation may also play a role in COPD. Cigarette smoke cytotoxicity in airway epithelial cells is mediated via activation of JNK (Lee et al., 2008).

The activation of JNK and AP-1 in models of asthma and COPD and its role in stimulating cytokine release and corticosteroid resistance suggests that JNK inhibitors may have therapeutic value in the treatment of asthma and COPD, which is supported by the beneficial effects of a selective JNK inhibitor SP600125 in these models. SP600125 is a competitive inhibitor of ATP for both JNK1 and JNK2. No clinical trials of JNK inhibitors have been undertaken, presumably because of toxicity issues.

Curcumin is a plant polyphenol found in turmeric, which inhibits JNK, and is currently in clinical trials for chronic inflammatory diseases, such as psoriasis; however, it is nonselective and targets several other signaling pathways, such as NF-kappaB and nuclear factor (erythroid-derived 2)-like 2 (Hatcher et al., 2008).

D. Extracellular Regulating Kinase/MAP Kinase Kinase Inhibitors

Several studies demonstrated activation of ERK1 and ERK2 in asthma and COPD models. Activation of mast
cells may release IL-1 and IL-6 through activation of ERK1/2, which is inhibited by genistein, a flavonoid that has additional pharmacological effects (Kim et al., 2014). IL-13 causes mucus hypersecretion as a result of increased MUC5AC via ERK activation (Kono et al., 2010). Epithelial growth factor receptor (EGFR)-induced mucus secretion is also mediated via ERK and inhibited by the ERK kinase (MEK1) inhibitor PD-98059 [2′-Amino-3′-methoxyflavone] (Li et al., 2012). Although p38 MAPK appears to be the dominant regulator of cytokine release from monocytes, in lung and alveolar macrophage ERK appears to be predominant and ERK inhibitors are more effective than p38 inhibitors in suppressing cytokine secretion (Koch et al., 2004; Tudhope et al., 2008). The ERK pathway is activated by growth factors and may be involved in proliferation of airway smooth muscle cells and other structural changes in the airways.

The proinflammatory effect of ERK suggest that an ERK inhibitor may be of potential benefit, particularly in reducing mucus secretion. The MEK1/ERK inhibitor PD-98059 is effective as an inhibitor of this pathway and acts as a noncompetitive allosteric inhibitor with a high degree of specificity. However, this compound is not suitable for in vivo or clinical studies because of its very low solubility. Several other MEK-1 inhibitors, such as trametinib and solumetinib, have been tested in clinical trials in cancer and have shown some efficacy in solid tumors and are relatively well-tolerated, but so far no studies in COPD or asthma have been reported (Wang et al., 2007; Fremin and Moloche, 2010). Interestingly, macrolide antibiotics inhibit cigarette smoke-induced CXCL8 secretion by epithelial cells, which is mediated via ERK phosphorylation, although macrolides have other effects on signaling such as inhibition of NF-κB (Shinkai et al., 2007).

E. Apoptosis Signal-Regulating Kinase-1 Inhibition

Apoptosis signal-regulating kinase-1 (ASK1) is an upstream kinase (MAP3K5) that activates both p38 MAPK and JNK and is activated by oxidative and endoplasmic reticulum stress (Hattori et al., 2009). Thioredoxin is normally bound to ASK1 to suppress its activity, and ASK becomes activated when thioredoxin is reduced by oxidative stress. ASK1 mediates the cytotoxicity of cigarette smoke in airway epithelial cells, which is inhibited by the closely associated antioxidant thioredoxin (Lee et al., 2008). ASK1 is a good potential target for COPD, and several small molecule inhibitors have been identified (Starosyla et al., 2015). Interestingly, ASK1 knockout mice are viable, whereas p38α and JNK knockout mice are embryonically lethal. Clinical trials are underway with ASK1 inhibitors, such as GS-4997, in diabetic renal disease (Lin et al., 2015), but so far these drugs have not been explored in COPD or asthma. Thioredoxin is the endogenous inhibitor of ASK1 but is difficult to deliver intracellularly.

F. TGFβ-Activated Kinase-1 Inhibition

TGFβ-activated kinase-1 (TAK1) is another upstream MAP kinase (MAP3K7) that is tightly regulated and activates both ERK and p38α MAPK pathways as well as NF-κB, thus playing a pivotal role in innate immunity in response to TGFβ, TNFα, IL-1β, and TLR via the adapter protein MyD88 (Sato et al., 2005; Ajbade et al., 2013). Proliferation of human airway smooth muscle in response to PDGF is significantly reduced by the selective TAK1 inhibition, with a dominant-negative inhibitor and small molecule inhibitor, 5Z-7-oxozeaenol (Pera et al., 2011). TAK1 inhibition also inhibits cigarette smoke-induced release of CXCL8 from airway smooth muscle cells (Pera et al., 2012). H. influenzae activates NF-κB and p38 MAPK in human airway epithelial cells via the activation of TAK1 and NIK (Shuto et al., 2001). TAK1 may also activate the adaptive immune system through the activation of NF-κB in T-lymphocytes (Sun et al., 2004). TAK1 is therefore an attractive target for inhibition, because both MAPK and NF-κB pathways would be suppressed. Several small molecule TAK1 inhibitors are in development (mainly for cancer therapy), but there are limited data on kinase selectivity and they have not yet advanced to clinical studies (Kilty and Jones, 2015).

G. MAPKAPK2 Inhibition

MAPKAPK2 (MK2) is activated directly via p38α MAPK, and inhibition of this downstream kinase might avoid some of the toxicity problems seen with p38α inhibitors (Gaestel, 2013). MK2 appears to play a role in prolonging NF-κB activation in Th2 cells and therefore amplifies allergic inflammation (Gorska et al., 2007). MK2 is also important in mediating the increased release of CXCL8 in response to cigarette smoke in human airway smooth muscle cells (Moreto et al., 2012). However, ATP-competitive MK2 inhibitors have poor selectivity and low solubility (Schlapbach and Huppertz, 2009). By contrast, noncompetitive inhibitors look more attractive and several compounds are in development but have not yet entered clinical trials (Anderson et al., 2009; Fiore et al., 2016).

VI. Phosphoinositide-3-kinase Inhibition

PI3Ks are a family of lipid kinases that generate lipid second messengers that regulate a number of cellular events, including innate and adaptive immune responses, growth, differentiation, cell motility and survival, and intracellular trafficking (Vanhaesebroeck et al., 2012; Hawkins and Stephens, 2015). PI3Ks catalyze the phosphorylation of the inositol ring of cell membrane phosphatidylinositols to generate lipid second messengers. They are classified into three classes based on the phosphorylated lipids generated. Class I PI3Ks catalyze the production of phosphatidylinositol-(3,4,5)-trisphosphate (PIP3) from PIP2 and are heterodimers comprising...
a p110 catalytic subunit with four isoforms (designated P110α, β, γ, and δ) together with an adaptor subunit of either p85 (class IA) that are triggered by receptor tyrosine kinases (RTKs) and include PI3Kα, β, and γ, or of p110/p84 (class IB) that are mainly triggered by G-protein-coupled receptors that include PI3Kγ (Vanhaesebroeck et al., 2016). PIP3 accumulates in the plasma membrane and recruits pleckstrin homology domain-containing proteins, including phosphoinositide-dependent kinase-1 (PDK1) and Akt (also called protein kinase B). PIP3-activated PDK1 then phosphorylates Akt at Thr308 site and, together with Ser473 phosphorylation by mammalian target of rapamycin complex 2 (mTORC2), this ensures full activation of Akt. P-Akt then triggers multiple downstream signaling pathways (Fig. 3). Downstream signaling from p-Akt is complex but a major target in mTORC1, which activates cellular senescence and is involved in accelerated aging (Johnson et al., 2013). PI3Kα and PI3Kβ are expressed in most cell types, whereas PI3Kγ and PI3Kδ are localized predominantly in leukocytes. By contrast, there is relatively little information about the function of Class II and III PI3Ks. Class II PI3Ks include PI3K-C2α, PI3K-C2β, and PI3K-C2γ. PI3K-C2α and PI3K-C2γ are the best characterized isoforms, and they appear to contribute to vesicular trafficking by regulating the production of PtdIns(3)P, but because there are no selective inhibitors they are difficult to investigate. Class III includes only one member, Vps34, which is involved in vesicular trafficking, TLR signaling, and autophagy via PtdIns(3)P generation.

The PI3K pathway is tightly regulated by phosphatases, particularly PTEN (phosphatase and tensin homolog deleted from chromosome 10), the major endogenous PI3K inhibitor, which is expressed in all cell types and dephosphorylates PIP3 to PIP2, thus turning off PI3K activation (Carracedo and Pandolfi, 2008; Worby and Dixon, 2014). PTEN acts as a tumor suppressor and deletions are commonly found in cancers such as lung cancer, but it is now recognized as an important regulator of inflammation and cell proliferation. The Src homology 2 domain-containing inositol 5'-phosphatase (SHIP) also inhibits PI3K signaling and is found mainly in hematopoietic cells, including macrophages (Backers et al., 2003).

A. Role in Asthma and Chronic Obstructive Pulmonary Disease

There is increasing evidence that PI3K plays a key role in asthma and COPD through activation of inflammation, corticosteroid resistance, and cellular senescence, resulting in accelerated aging (Fig. 3).
(Marwick et al., 2010b). These effects are mediated via Class I PI3Ks, and the potential role of Class II and II PI3Ks is unknown. A nonselective PI3K inhibitor LY294002 [2-Morpholin-4-yl-8-phenylchromen-4-one] given by inhalation inhibits the allergic inflammatory response to allergen in sensitized mice, with reduction in eosinophils, Th2 cytokines, and airway hyperresponsiveness (AHR) (Duan et al., 2005a). Similar results were obtained with a dominant-negative inhibitor of the regulatory subunit p85, indicating that Class IA PI3Ks are involved and may determine the switch from Th1 to Th2 cytokines (Myou et al., 2003). More specifically PI3Kδ plays a key role in mast cell degranulation after cross-linking of IgE receptors, an effect blocked by inactivation of PI3Kδ and by the selective PI3Kδ inhibitor IC87114 (Ali et al., 2008; Kim et al., 2008). In the murine model of asthma, intratracheal administration of IC87114 suppressed the allergic response, Th2 cytokines, and AHR (Lee et al., 2006a), and these effects are mirrored in PI3Kδ-inactivated mice (Nashed et al., 2007). In an Aspergillus fumigatus model of allergic inflammation in mice, PI3Kδ inhibition suppressed the allergic response, and this effect was mediated through inhibition of endoplasmic reticulum stress, which is increased in asthma and results in NF-κB activation (Lee et al., 2016). PI3Kδ may also play a role in increasing contractility of canine airway smooth muscle (Halayko et al., 2004) and the increased proliferation of airway smooth muscle in response to growth factors such as TGF-β (Goldsmith et al., 2006).

PI3K activation is also important in COPD. Total PI3K activity measured as p-Akt is markedly increased in peripheral lungs and macrophages of patients with COPD, and there is increased expression of the PI3Kδ isoform (Marwick et al., 2009; To et al., 2010). In vitro oxidative stress increases p-Akt in peripheral blood monocytes and alveolar macrophages, which is prevented by a PI3Kδ but not PI3Kγ inhibitor (Marwick et al., 2010a). PI3Kγ knockout mice show reduced neutrophil migration and activation, as well as impaired T-lymphocyte and macrophage function (Medina-Tato et al., 2007), and PI3Kγ is the pivotal signaling pathway mediating neutrophil recruitment to the lung after instillation of chemokines (Thomas et al., 2005). PI3Kγ plays an important role in chronic inflammatory and immune diseases (Costa et al., 2011). PI3Kγ is also involved in the inflammatory response and may cooperate with PI3Kδ in the activation of neutrophils, for example (Condiffe et al., 2005). Both PI3Kγ and PI3Kδ inhibitors improve the accuracy of migration in COPD neutrophils (Sapey et al., 2014). An aerosolized PI3Kγδ inhibitor [TG101-115 (3-2,4-diamino-6-(3-hydroxyphenyl)pteridin-7-yl)phenol (6,7-Bis(3-hydroxyphenyl)pteridine-2,4-diamine)] was effective in cigarette smoke-induced pulmonary inflammation in mice, which is corticosteroid resistant, and also in the allergic inflammation model (Doukas et al., 2009). PI3Kδ is also involved in corticosteroid resistance after oxidative stress, which is mediated by reduced histone deacetylase-2 (HDAC2), and PI3Kδ inhibitors may reverse corticosteroid resistance in patients with COPD by increasing HDAC2 expression. Inhibition of PI3Kδ with IC87114 reverses corticosteroid-resistance to cigarette smoke exposure in mice (To et al., 2010), and PI3Kδ-inactivated mice do not develop corticosteroid-resistance after cigarette smoke exposure, whereas PI3Kγ-inactivated animals develop corticosteroid-resistant inflammation as normal (Marwick et al., 2009). PI3Kα is involved in cell proliferation and survival and is activated in early lung cancer cells and so may contribute to the increased risk of developing lung cancer in patients with COPD. PI3K activation in COPD also activates mTOR, measured by phosphorylation of p70 ribosomal S6-kinase (S6K), which is increased in COPD lungs and PBMC (Mitani et al., 2015). S6K not only reduces HDAC2 but also leads to activation of JNK, increased c-Jun, and activation of AP-1, which also contributes to corticosteroid resistance. PI3K activation also drives cellular senescence and accelerated aging in COPD lungs through the activation of mTOR, which plays a pivotal role in accelerated aging (Johnson et al., 2013; Mercado et al., 2015). This leads to a reduction in the key antiaging molecule sirtuin-1, which is reduced in the lungs of patients with COPD (Rajendrasozhan et al., 2008; Nakamaru et al., 2009). The same PI3K-mTOR pathway may also lead to the comorbidities associated with COPD, such as ischemic heart disease, type 2 diabetes, osteoporosis, chronic renal disease, and dementia (Barnes, 2015a). For example, endothelial progenitor cells, which are important for repairing damage to endothelial cells to maintain normal vascular function, show accelerated aging and decreased sirtuin-1 due to activation of PI3K (Paschalaki et al., 2013).

B. Inhibiting Phosphoinositide-3-kinase-Akt-Mammalian Target of Rapamycin Signaling

In view of the critical role of PI3K-Akt-mTOR signaling it is not surprising that there has been an intense effort to discover drugs that inhibit this pathway. This has been mainly in the field of cancer in view of the key role of PI3K in cell proliferation, and the PI3K-Akt-mTOR pathways are the most frequently dysregulated in human solid tumors. The pathways are also relevant to chronic degenerative diseases that involve accelerated aging (Johnson et al., 2013) and in the treatment of inflammatory and immune diseases (Hawkins and Stephens, 2015). There has been great interest in the potential for inhibitors of the PI3K pathways in the treatment of severe asthma and COPD (Ito et al., 2007).

1. Phosphoinositide-3-kinase Inhibitors. Nonselective inhibitors of Class I PI3K include wortmannin and
LY294002, which are useful in in vitro and in vivo animal studies but are not suitable for clinical use because of their poor pharmacokinetics and high toxicity. Several pan-class I PI3K inhibitors have been developed for use in malignancy, including hematologic malignancies and solid tumors, but so far none have been approved for clinical use, mainly because of toxicity issues (Dienstmann et al., 2014). This has led to a search for isoform-selective inhibitors. However, PI3K isoforms may compensate for each other, at least in cancer, through feedback mechanisms, where blocking one isoform leads to a compensatory increased in the noninhibited isoforms. For example a PI3Kβ-selective inhibitor leads to an increase in PI3Kδ (Schwartz et al., 2015). PI3Kα inhibitors have been developed for treating solid tumors but may result in hyperglycemia, because they interfere with insulin signaling. PI3Ky inhibitors have been considered, but so far no drugs have been developed for clinical use (Ruckle et al., 2006) as potential anti-inflammatory treatments (Ameriks and Venable, 2009). PI3Kδ inhibitors have been developed for the treatment of B cell malignancies and the PI3Kδ-selective inhibitor idelalisib (CAL-101) has been approved for the treatment of some B cell malignancies (Vanhaesebroeck and Khwaja, 2014). However, in the treatment of chronic lymphocytic leukemia, side effects such as leukopenia, colitis, and skin rashes may develop with idelalisib (Brown, 2015; Louie et al., 2015). There is a good rationale for combining inhibition of PI3Kδ and δ, and several combined PI3Kγ/δ inhibitors are in development, such as IPI-145 (duvelisib), which is effective in several inflammation and autoimmune animal models and is now in early clinical development (Winkler et al., 2013). To overcome dose-limiting side effects for the treatment of asthma and COPD, these inhibitors have been developed for inhaled delivery. A combined PI3Kγ/δ inhibitor TG100-115 is effective in models of allergic asthma and cigarette smoke-induced inflammation in mice and is not in clinical development (Doukas et al., 2009). There is a good rationale for this combination because PI3Kγ inhibition should be anti-inflammatory and inhibit cell trafficking into the lungs, whereas PI3Kδ inhibition should be effective in reversing corticosteroids resistance in COPD and severe asthma. Inhaled PI3Kδ inhibitors, such as GS4286 (2-[4-tert-butyl-3-(trifluoromethyl)phenyl]-6-(7-cyano-2-oxo-2H-chromen-3-yl)-1H-pyrimidin-4-yl)acetic acid, are also in development for the treatment of COPD and severe asthma (Wilson et al., 2013). Another approach to inhibition of PI3Kδ is the use of existing therapies. Theophylline was shown to increase HDAC2 and reverse corticosteroids resistance in macrophages form COPD patients (Ito et al., 2002; Cosio et al., 2004) and is effective through the selective inhibition of oxidant-activated PI3Kδ in relatively low concentrations that are lower than those used for its bronchodilator effect (To et al., 2010). A clinical trial of low-dose theophylline combined with inhaled corticosteroids is now underway in COPD patients (Barnes, 2013c; Devereux et al., 2015). Nortriptyline, a tricyclic antidepressant, and solithromycin, a novel macrolide antibiotic, are also effective in reversing corticosteroid-resistance in COPD cells through inhibiting PI3Kα (Mercado et al., 2011a; Kobayashi et al., 2013).

2. Akt-Mammalian Target of Rapamycin Inhibitors. Akt is a downstream target of PI3K activation, and several oral inhibitors have been developed for use in cancer therapy. Perfosine blocks p-Akt and is in several clinical trials for malignancy, but this drug appears to also target several other pathways, leading to the development of more selective inhibitors (Alexander, 2011). Rapamycin (sirolimus) is a macrolide that binds to FK506-binding protein-12, which functions as an allosteric inhibitor of mTORC1. It is already in clinical use as an immunosuppressant in transplantation and autoimmunity, although it has frequent and severe adverse effects that limit its chronic use. There is particular interest in finding less toxic analogs of rapamycin with improved pharmacokinetics (rapalogs), because rapamycin extends life span in mammals by targeting cellular senescence and autophagy (Wilkinson et al., 2012; Lamming et al., 2013). Indeed, a recent clinical study suggests that a rapalog everolimus is able to reduce immunosenescence in elderly people (Mannick et al., 2014). Results in cancer therapy have been disappointing (Chiariini et al., 2015), and this may reflect the fact that blocking mTOR, which reduces S6K activation, results in blocking of a feedback inhibitory loop that results in PI3K-Akt activation in malignant cells. The reduction in autophagy by rapamycin may also work against the killing of malignant cells. Dual inhibitors of mTORC1 and 2, such as AZD2014 [3-(2,4-bis((S)-3-methylmorpholino)pyrido [2,3-d]pyrimidin-7-yl)-N-methylbenzamide], may be more effective because this blocks the feedback loop where mTORC2 activates Akt (Dienstmann et al., 2014). This has suggested that dual inhibitors of PI3K and mTOR may be more beneficial, because this avoids the PI3K-Akt feedback loop. There is close structural homology between these kinases, and several dual inhibitors have been developed and are now in clinical trials (Chiariini et al., 2015). None of these treatments has so far been tested in airway disease. However, rapamycin is very effective in inhibiting mTOR activation in COPD lung and PBMC, which results in reduced corticosteroids resistance (Mitani et al., 2015). In addition, rapamycin is effective in suppressing allergic inflammation in sensitized mice and inhibits eosinophil differentiation (Mushaben et al., 2013; Hua et al., 2015). Interestingly, rapamycin switches the phenotype of lymphocytes from Th2 to regulatory T cells (Foxp3⁺) (Kim et al., 2010).

3. Activating Endogenous Inhibitors. Adenosine monophosphate-activated kinase (AMPK) is an important regulator of PI3K signaling through the inhibition
of mTORC1 via phosphorylation of the tumor suppressor complex TSC1/2 and plays an important role in the regulation of metabolism, cell energetics, and mitochondrial function, but may also have antiaging effects through increasing sirtuin-1 and anti-inflammatory effects (Steinberg and Kemp, 2009). This has prompted a search for AMPK activators, which should have value in asthma and COPD (Scarpulla, 2011; Salt and Palmer, 2012). Metformin, a biguanide, is commonly used to treat type 2 diabetes and works partly through activation of AMPK via changes in nucleotide levels. Experimentally it has been shown to extend life span in mice (Martin-Montalvo et al., 2013). It also has potential as an anti-inflammatory drug (Salt and Palmer, 2012). Metformin and the more specific AMPK activator 5’-aminoimidazole-4-carboxamide-1-β-4-ribofuranoside reduce allergic inflammation in a chronic allergic mouse model, and this is reproduced in AMPK-deficient (AMPKα1−/−) mice (Park et al., 2012). 5’-Aminoimidazole-4-carboxamide-1-β-4-ribofuranoside inhibits cigarette smoke-induced release of CXCL8 from A549 cells, and AMPKα1−/− mice have reduced emphysema after cigarette smoke exposure, indicating that activation of this pathway has therapeutic potential in asthma and COPD (Lee et al., 2015). Flavones, such as quercitin, and resveratrol also activate AMPK. Resveratrol has anti-inflammatory effects in COPD airway epithelial cells, although this may be mediated through several mechanisms (Donnelly et al., 2004). More direct activators of AMPK are now in development, such as the compound A-769662, which is entering clinical trials for diabetes (Goransson et al., 2007).

Another approach is through activation of the phosphatases PTEN and SHIP, which inhibit PI3K signaling. It has been difficult to identify selective activators of PTEN, which may reflect the complexity of its regulation and may interactions (Song et al., 2012). SHIP is more amenable to activation and a small molecule activator of SHIP-1, AQX-1125, and is effective in reducing p-Akt and reducing mediator release, mast cell activation, and leukocyte chemotaxis in vitro (Stenton et al., 2013b) and inflammation in allergen-induced and cigarette smoke-induced pulmonary inflammation in mice in vivo (Stenton et al., 2013a). A recent clinical trial of oral AQX-1125 in patients with mild asthma showed a significant reduction in later response after allergen challenge and a (nonsignificant) reduction in inflammatory biomarkers (Leaker et al., 2014). The drug is well tolerated, suggesting that it might be a potential treatment of asthma and COPD.

Another downstream inhibitor of PI3K signaling pathways is glycogen synthase-3β (GSK3β), which is constitutively active but inhibited by phosphorylation by Akt and ERK1/2 as a result of oxidative stress (Takahashi-Yanaga, 2013). Inactivated GSK3β (p-GSKβ-Serγ) is increased in PBMC, macrophages, and airway epithelial cells of patients with COPD, especially those with severe disease (Ngkelo et al., 2015). This is associated with corticosteroid resistance as a result of reduced HDAC2 in these cells, which is mimicked by GSK3β knockdown and a GSK3β inhibitor CT99021 [6-[[2-[[4-(2,4-Dichlorophenyl)-5-(5-methyl-1H-imidazol-2-yl)-2-pyrimidinyl]amino]ethyl]amino]-3-pyridinecarbonitrile]. This suggests that GSK3β activators may be beneficial in reversing corticosteroid resistance, but it has been difficult to identify such compounds. In addition, GSK3β regulates many different signaling pathways, including NF-κB, and its activation might amplify inflammation.

VII. Janus-Activated Kinase Inhibition

The Janus kinase (JAK)-signal transducer of transcription (STAT) signaling pathway regulates the expression of multiple inflammatory and immune proteins and has been implicated in many inflammatory diseases (O’Shea et al., 2015). This has led to the development of JAK inhibitors (Jakinibs), which are a new class of anti-inflammatory drug now used for the treatment of inflammatory diseases, such as rheumatoid arthritis, autoimmune diseases, hematopoietic diseases, and cancer, with several inhibitors in clinical development and some already in clinical use (Kontzias et al., 2012; Menet et al., 2013). After occupation of the cytokine receptor, the associated JAKs become activated and both phosphorylate each other and the intracellular tail of their receptors, thus creating docking sites for cytoplasmic STATs, which in turn directly bind as dimers to DNA and regulate gene expression. There are four JAKs (JAK1-3) and Tyk2, which selectively bind different receptor chains (Fig. 4). The selective usage of JAKs by different receptors explains their distinct in vivo roles. There are seven STATs (STAT1, 2, 3, 4, 5a, 5b, 6) that are activated by different combination of JAKs, but JAKs may also directly affect genes through histone phosphorylation, which changes chromatin structure to influence gene expression. Various polymorphisms of STAT-encoding genes have been described in cancer and inflammatory and immune diseases.

A. Role in Asthma and Chronic Obstructive Pulmonary Disease. There is recent evidence that JAKs are involved in asthma and COPD. Many of the cytokines involved in asthma, including the Th2 cytokines (IL-4, IL-5, IL-9, and IL-13), GM-CSF, and thermic stromal lymphopoietin, activate JAK-STAT signaling (Pernis and Rothman, 2002; Ghoreschi et al., 2009). The receptors for these cytokines involve activation of all of the JAKs, suggesting that a broad spectrum inhibitor is more likely to be useful. JAK3 expression is restricted to hematopoietic cells and colocalizes with JAK1, transmitting signals through the common γ-chain of the cytokine receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. Tofacitinib and pyridone-6, which are pan-JAK
inhibitors, are effective and reduce AHR in a murine model of allergic inflammation (Kudlacz et al., 2008; Matsunaga et al., 2011). There has been concern that blocking JAK2 may have inhibitory effects on hematopoiesis, so there has been a search for more selective inhibitors. JAKs play a key role in differentiation of Th2 cells and a JAK1/3 inhibitor (R256) blocked the differentiation of Th2 cells and the phosphorylation of STAT5 and STAT6, but not of Th1 or Th17 cells in vitro (Ashino et al., 2014). In vivo R256 inhibited allergic inflammation, AHR, and mucus hypersecretion in a mouse model without any changes in Th2 cytokine concentrations.

JAKs are also involved in the mechanisms of COPD and several cytokines implicated in COPD activate JAK-STAT signaling, including IL-6, IL-12, and interferon (IFN)-γ, which activate JAK1, JAK2, and Tyk2. In mice with hyperreactivity of the receptor for IL-6 (gp130), there was an increase in STAT3 and pulmonary inflammation that were prevented in STAT3 knockout mice (Ruwanpura et al., 2012). Furthermore, STAT3 was increased in the lungs of COPD patients and correlated with inflammation but not with airway obstruction. STAT1 and STAT3 are phosphorylated in the lungs of severe COPD patients to a greater extent than in smokers and nonsmokers, indicating activation of several JAKs (Yew-Booth et al., 2015). STAT4 is also increased in the airway of COPD patients, presumably in response to IL-12 or IL-23 (Di Stefano et al., 2004). The chemokines CXCL9, CXCL10, and CXCL11 are all increased in the airways of COPD patients and target a common receptor, CXCR3, which is involved in the recruitment of T-cells (particularly CD8+ cells) into the lung (Costa et al., 2008). These chemokines are induced by IFNγ through the activation of JAKs and phosphorylation of predominantly STAT1 (Tudhope et al., 2007). Pan-JAK inhibitors are effective in reducing IFNγ-induced secretion of CXCR3-activating chemokines from human airway epithelial cells, demonstrating their potential as anti-inflammatory treatments in COPD (Fenwick et al., 2015).

B. Jakinibs. There is a good rationale for the use of JAK inhibitors in both asthma and COPD, because they mediate the effects of several mediators involved in these diseases. Several oral JAK inhibitors (jakinibs) are now approved and in clinical trial for the treatment of other inflammatory diseases, including rheumatoid arthritis, inflammatory bowel disease, and psoriasis (Kontzias et al., 2012; Menet et al., 2013; Schwartz et al., 2016). Over 20 pan-JAK inhibitors and isoform-selective inhibitors have now been developed. Tofacitinib is JAK1/3 selective and indicated for rheumatoid arthritis and psoriasis, whereas JAK2-selective inhibitors are indicated for hematologic malignancies and myeloproliferative disease. Ruxolitinib is JAK1/2 selective and indicated for rheumatoid arthritis and myeloproliferative disease. However, the selective JAK inhibitors are not specific so tend to affect other JAKs, increasing the risk of side effects. Thus pan-JAK inhibitors may cause anemia, neutropenia, and thrombocytopenia through inhibition of JAK2, which mediates the effects of erythropoietin, thrombopoietin, and GM-CSF. Side effects of jakinibs after systemic administration are dose limiting and include increased infections, diarrhea, increased cholesterol levels, and lymphomas, so a strategy to reduce these adverse effects...
effects is to deliver the drugs topically. Thus, topical tofacitinib and ruxolitinib have been developed for skin application for the treatment of psoriasis. Inhaled pan-JAK inhibitors, such as LAS194046 and R256, are now in development for asthma and COPD and have shown efficacy in models of allergic asthma (Ramis et al., 2014). An inhaled pan-JAK inhibitor (VR560) markedly reduced the allergic inflammatory response in mice sensitized and exposed to chronic house dust mites, inhibited Th2 cytokines and IL-17, and inhibited STAT3 phosphorylation (Wiegman et al., 2015). This suggests that inhaled pan-JAK inhibitors may have good potential in asthma and COPD and clinical trials are now in progress.

VIII. Spleen Tyrosine Kinase Inhibition

Spleen tyrosine kinase (Syk) is a nonreceptor cytoplasmic protein tyrosine kinase that is primarily expressed in hematopoietic cells (particularly B lymphocytes) and links immune cell receptors to multiple intracellular signaling pathways that regulate many cellular responses, including cytokine release and cell survival (Mocsai et al., 2010; Riccaboni et al., 2010). Syk appears to have many diverse functions, including innate and adaptive immunity, cell adhesion, and organ development and has been implicated in many autoimmune, inflammatory, and malignant diseases. Immune receptors include B-cell and T-cell receptors and Fc-receptors, which associate with transmembrane proteins that have immunoreceptor-tyrosine-based activation motifs (ITAMs), which are short peptide sequences containing two tyrosine residues 6–12 amino acids apart. ITAM tyrosines are phosphorylated after ligand binding, resulting in the recruitment and activation of Syk or the related enzyme ζ-activated protein kinase of 70 kDa, which results in recruitment of other kinases and the activation of downstream signaling pathways, including NF-κB and PI3K pathways (Fig. 5).

A. Role in Asthma and Chronic Obstructive Pulmonary Disease. In a chronic allergic murine model of asthma there was an increase in Syk activation in epithelial cells, and a Syk inhibitor \([\text{N}-(4-[6-(cyclobutylamino)-9H-purin-2-yamino]phenyl]-N-methylacetamide (NVP-QAB-205)]\) significantly reduce AHR after allergen and the enhanced AHR after additional exposure to diesel particulates and ozone, although there was no reduction in inflammatory cells (Penton et al., 2013). A Syk inhibitor \([6-(5-fluoro-2-(3,4,5-trimethoxyphenylamino) pyrimidin-4-ylamino)-2,2-dimethyl-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (R406)]\) also prevents activation of sensitized mast cells and dendritic cells from mice, with a reduction in AHR and allergic inflammation in vivo (Matsubara et al., 2006a,b). Similar effects are seen with an inhaled Syk antisense, indicating the specificity of the small molecule Syk inhibitors (Stenton et al., 2000, 2002). An oral inhibitor \([2-(7-(3,4-dimethoxyphenyl)-imidazo[1,2-c]pyrimidin-5-ylamino)-nicotinamide HCl (Bay 61-3606)]\) is also effective in reducing the allergic reaction to inhaled allergens in rats and also in preventing passive cutaneous anaphylaxis and mast cell activation (Yamamoto et al., 2003). This Syk inhibitor also inhibits the activation of human basophils. The reduction in AHR seen after a Syk inhibitor may be due to an effect on airway smooth

![Fig. 5. Spleen tyrosine kinase signaling. Syk is activated by immune receptors via phosphorylation by Lyn kinase and may activate PI3K or phospholipase C_\(\gamma\), resulting in activation of immune cells, such as mast cells, B-lymphocytes, and T-lymphocytes. Syk may also be activated by integrins. Several Syk inhibitors have been identified (shown in green box).](image-url)
muscle contractility, because Syk-deficient mice do not develop AHR after allergens and the contractility of small airways to spasms is reduced (Wang et al., 2015). This is mimicked by treatment with a Syk inhibitor (Moy et al., 2013). Syk is also activated by infection of human airway epithelial cells after rhinovirus binding to ICAM-1 and results in the activation of p38 MAPK and PI3K activation, with release of mediators such as CXCL8 and VEGF and so may play a role in acute exacerbations of both asthma and COPD (Lau et al., 2008). Syk also regulates internalization of rhinovirus via clathrin-mediated endocytosis, which involves ITAMs previously only associated with immune cells (Lau et al., 2011).

B. Spleen Tyrosine Kinase Inhibitors. Because of the key role of Syk in mast cell and immune cell signaling, the effects of Syk inhibition on the allergic inflammatory response, AHR, and the inhibition of rhinovirus signaling there is a persuasive case for development of Syk inhibitors (Geahlen, 2014). Several orally active Syk inhibitors have been developed for the treatment of rheumatoid arthritis and B cell leukemias (Norman, 2014b; Thorarensen and Kaila, 2014) For example, fostamatinib (R788) has shown efficacy in rheumatoid arthritis patients (but not those refractory to biologics), and the most frequent adverse effects are gastrointestinal symptoms, hypertension, and neutropenia, but no serious infections (Nijjar et al., 2013). To avoid side effects, topical formulations have been developed. A nasal spray formulation of R112 was weakly effective in patients with seasonal allergic rhinitis exposed to outdoor allergens (Meltzer et al., 2013) and transiently decreased the concentration of PGD2 in nasal lavage after nasal allergen challenge but had no effect on either histamine or mast cell tryptase (Guyer et al., 2006). A novel Syk inhibitor suitable for inhalation, LA5183986 [1-(2,2-diarylaminoethyl)-3-(pyridin-4-yl)-N-pyrazin-2-yl-1H-indazol-3-amine], delivered to ovalbumin-sensitized Brown Norway rats showed good inhibition of the early response to allergen and of mast cell degranulation, without any effects on systemic mast cells (Ramis et al., 2015), and so is in clinical development for asthma.

IX. Receptor Tyrosine Kinases

Growth factors are cytokines that induce structural changes, such as fibrosis and tissue remodeling, and act on high-affinity surface receptors that have tyrosine kinase activity. Of the 90 human protein tyrosine kinase genes, over half encode receptor tyrosine kinases (RTK) (Hubbard and Till, 2000). RTKs are important in normal tissue growth, development, and repair after injury and have been closely linked to cancers. Most RTKs function as single unit receptors, although some (e.g., insulin receptor) are multimeric. An N-terminal extracellular domain interacts with the protein ligand (growth factor or hormone), and the C-terminal intracellular domain contains a relatively well-conserved domain that catalyzes receptor autophosphorylation with receptor dimerization and phosphorylation of RTK substrates, which include proteins with Src homology-2 domains and phosphotyrosine domains (Lemmon and Schlessinger, 2010). There are several distinct families with RTKs, but those most relevant to airway disease are the epidermal growth factor receptor (EGFR, ErbB) family, fibroblast growth factor receptor (FGFR) family, vascular-endothelial growth factor receptor (VEGFR) family, and platelet-derived growth factor receptor (PDGFR).

A. Role in Asthma and Chronic Obstructive Pulmonary Disease

EGFR play an important role in mediating mucus hypersecretion and mucus metaplasia, with goblet cell proliferation in both asthma and COPD (Nadel and Burgel, 2001). EGFR shows increased expression in airway epithelial cells of asthmatic airways and is associated with CXCL8 release and neutrophilic inflammation (Hamilton et al., 2003). EGFR plays a pivotal role in mucus hypersecretion (chronic bronchitis) in COPD through increased expression of MUC5AC and may be activated by ligand such as TGF-α that may be generated by neutrophilic inflammation and through nonligand mechanism such as oxidative stress (Takeyama et al., 2000; Takeyama et al., 2001; Shao and Nadel, 2005). There is increased expression of EGFR in airway epithelia cells of COPD airways (de Boer et al., 2006).

Basic fibroblast growth factor-2 (bFGF) also stimulates airway smooth muscle proliferation and acts in concert with other growth factors (Bosse and Rola-Pleszczynski, 2006). Chronic allergen exposure in sensitized mice induces bFGF expression in airway macrophages and is associated with airway remodeling, both of which were reduced after treatment with a corticosteroid (Yum et al., 2011).

Increased angiogenesis contributes to the structural remodeling in asthma, with increased numbers of bronchial vessels, which show increased leakiness (Paredi and Barnes, 2009). These effects are largely mediated by VEGF secreted from airway epithelial cells and airway smooth muscle cells, T cells and eosinophils (Hoshino et al., 2001). There is increased expression of VEGF, VEGF receptors, and angiogenin-1 in airways of asthmatic patients that correlates with increased vascularity (Feltis et al., 2006). By contrast, VEGF levels are reduced in COPD sputum and lung parenchyma (Taraseviciene-Stewart et al., 2006; Kanazawa et al., 2003) and may be linked to the development of emphysema through apoptosis of endothelial cells. VEGF levels are reduced in the epithelia lining fluid of peripheral airways of patients with COPD and normal smokers (Kanazawa et al., 2014).
The platelet-derived growth factor (PDGF) family includes the isoforms A-D, which may exist as homo- or heterodimers and activate the receptors PDGFR-α and -β, which dimerize. (PDGF)-BB is a potent stimulator of airway smooth muscle proliferation (Hirst et al., 1996) and may act synergistically with other growth factors such as endothelin-1 (Yahiaoui et al., 2006). The proliferative effect of PDGF is mediated via activation of STAT-3 and the small GTPase Rac-1 (Simeone-Penney et al., 2008). Overexpression of PDGF-BB in airway epithelial cells induces airway smooth muscle (ASM) proliferation and airway hyperresponsiveness in a mouse model (Hirota et al., 2011). Chronic allergen exposure induces secretion of PDGF into BAL fluid in this model.

B. Receptor Tyrosine Kinase Inhibitors

Several small molecule EGFR inhibitors, such as gefitinib and erlotinib, have been developed for the treatment of solid cancers, including lung cancers, where mutations of EGFR are commonly seen. Experimental studies with EGFR reduces mucus secretion. An EGFR inhibitor AG1478 [N-(3-chlorophenyl)-6,7-dimethoxy-4-quinazolinamine] is effective at inhibiting MUC5AC secretion induced by LPS, TNF-α, or cigarette smoke from human airway epithelial cells in vitro and in cigarette smoke-induced MUC5AC secretion in rats in vivo (Hegab et al., 2007; Takezawa et al., 2016). However, a potent and selective EGFR inhibitor BIBW 2948 given by inhalation for 4 weeks in patients with COPD failed to show any reduction in epithelial mucus, despite evidence for target engagement measured by EGFR internalization (Woodruff et al., 2010). In addition, there was a reduction in lung function, and liver enzyme elevation was seen in some patients. VEGFR inhibitors, such as cediranib, have been developed for certain solid cancers but have not been tested in asthma and would be contraindicated in COPD because experimental VEGFR inhibitors have induced emphysema. Several drugs inhibit PDGFR and usually inhibit other tyrosine kinases. For example, masitinib targets PDGFR and c-Kit and tyrosine kinases, improves asthma control in severe asthma patients, and reduces the requirement for oral corticosteroids, although it was associated with skin rashes and edema (Humbert et al., 2009). Nintedanib inhibits PDGFR, but also VEGFR and FGFR, and has been approved for pulmonary fibrosis but has not been studied in airway disease (Keating, 2015). Similarly, imatinib inhibits PDGFR and c-Kit, as well as ABL-kinase, the key target in chronic myeloid leukemia, but has not been studied in asthma or COPD.

C. c-Kit Inhibitors

c-Kit (also known as CD117 and SCFR) is a proto-oncogene, a tyrosine kinase receptor for stem cell factor, which is an essential mast cell growth factor. It is encoded by the KIT gene and expressed on hematopoietic progenitor cells and mature mast cells (Lennartsson and Ronnstrand, 2012). Activating mutations of KIT are associated with mastocytosis, with an accumulation of mast cells in tissues. Silencing of c-Kit with a small interference RNA reduces the inflammatory response to allergen in a murine model of asthma, with a reduction in Th2 cytokines and eosinophils (Wu et al., 2012). Imatinib, an ABL-kinase inhibitor for chronic myeloid leukemia, is also an inhibitor of c-Kit tyrosine kinase activity and is effective in a mouse model of asthma with hypersensitivity to cockroach allergen (Berlin and Lukacs, 2005). This suggests that more selective c-Kit inhibitors may be useful in the treatment of asthma (Reber et al., 2006). As discussed above, masitinib inhibits c-Kit and has produced benefits in asthma. Other receptor tyrosine kinases, such as sunitinib and nilotinib, have been developed for cancer therapy and may also be effective but have dose-limiting side effects (von Mehren, 2006).

X. Transforming Growth Factor-β Receptor Kinase

Transforming growth factor(TGF)-β is a member of a large superfamily, which includes bone morphogenic proteins, activins, and inhibins. It plays an important role in tissue repair, proliferation, and cell differentiation (Massague, 2012). It is implicated in several chronic lung diseases, including asthma and COPD, where it is involved in tissue remodeling (Aschner and Downey, 2016). It is often associated with tissue fibrosis through the induction of the growth factor connective tissue growth factor (also known as CCN2) (Ihn, 2002). TGF-β also plays an important role in immunoregulation and is secreted by subtypes of regulatory T cells (Levings et al., 2002). There are three isoforms of TGF-β (TGF-β1, TGF-β2, TGF-β3), secreted noncovalently bound to latency-associated peptide, which dissociate to release active TGF-β with acidification, oxidative stress, certain integrins, and the action of MMP-2 and MMP-9, so that TGFβ is activated at sites of inflammation (Yu and Stamenkovic, 2000). Activated TGF-β binds to activate TGF-β type I (T-βRI) via phosphorylation by T-βRII, which has Ser/Thr kinase activity. Phosphorylation of T-βRII then recruits Smad proteins, enabling Smad phosphorylation and nuclear translocation, resulting in activation of various genes. There are eight Smad proteins in humans, including receptor-Smads (1, 2, 3, 5, 8), co-Smad4, and inhibitory Smads (6, 7). TGF-β signaling may also be activated in a Smad-independent manner by MAP kinases, PI3K and Src-family kinases.

A. Role in Asthma and Chronic Obstructive Pulmonary Disease

TGF-β plays a key role in the airway remodeling of asthma (Aschner and Downey, 2016). TGF-β1 levels are increased in asthmatic airways and there is increased TGF-β in the submucosa of patients with asthma (Kohturk et al., 2003). TGF signaling may promote...
ECM deposition, airway smooth muscle proliferation, and collagen deposition after allergen challenge. TGF-β may be derived from eosinophils, mast cells, macrophages, epithelial cells, or fibroblasts (Minshall et al., 1997; Halwani et al., 2011). It is increased particularly in severe asthma (Balzar et al., 2005). TGF-β induces proliferation of human airway smooth muscle cells via activation of multiple kinases, including PI3K, ERK, JNK and by NF-κB activation (Goldsmith et al., 2006).

There is increased expression of TGF-β in epithelial cells from small airways of COPD patients (Takizawa et al., 2001; Wang et al., 2008) and this may be compounded by downregulation of inhibitory Smads (Smad6, Smad7) as a result of exposure to cigarette smoke (Springer et al., 2004). TGF-β is also expressed in macrophages in the walls of small airways of patients with COPD (de Boer et al., 1998). Oxidative stress is a potent stimulus to increased TGF-β activation and expression (Koli et al., 2008) and TGF-β is activated from its latent precursor by MMP-9, which is secreted in COPD (Yu and Stamenkovic, 2000; Dallas et al., 2002).

### B. T-βRI Kinase Inhibitors

Several small molecule inhibitors of T-βRI kinase have been developed, such as SB-431542 [4-[2-(1,3-benzodioxol-5-yl)-5-(2-pyridinyl)-1H-imadazol-2-yl]benzamid] and SB-505154 [2-(5-benzo[1,3]dioxol-5-yl-2-tert-butyI-3H-imadazol-4-yl)-6-methylpyridine hydrochloride], which inhibit TGF-β signaling and Smad activation (Laping et al., 2002; DaCosta Byfield et al., 2004). For example, these drugs are effective in the bleomycin model of lung fibrosis (Koh et al., 2015). In a rat model of chronic allergen exposure, a T-βRI kinase inhibitor, SD-208 [2-(5-chloro-2-fluorophenyl)pteridin-4-yl]pyridin-4-yl-amine], reduced airway hyperresponsiveness and collagen deposition, but there was no reduction in airway smooth muscle proliferation (Leung et al., 2006). However, a T-βRI kinase inhibitor was effective in reducing human ASM proliferation in vitro (Xie et al., 2007). Clinical development of these inhibitors has proven problematic because of adverse effects, so no clinical trials appear to have been conducted. A T-βRI kinase inhibitor, galunisertib, was entered into clinical trials for cancers but had marked cardiac toxicity that has slowed further development (Herbertz et al., 2015). Inhibiting TGF-β signaling may have unwanted effects through inhibiting regulatory T cells activity, so that targeting fibrosis may be better achieved by targeting the downstream target connective tissue growth factor (Jun and Lau, 2011).

### XI. Protein Kinase C Inhibition

Protein kinase C (PKC) is a family of protein kinases that regulate a variety of downstream proteins via Ser/Thr phosphorylation. PKCs are activated by diacylglycerol, generated via phospholipase C activation, by phospholipids, or by calcium ions to regulate signal propagation within cells (Rosse et al., 2010). There are 15 subtypes of PKC, organized into three main families, determined by the method of activation. On activation, PKCs translocate from the cytoplasm to the cell membrane, where they remain activated for a long time and mediate myriad functions, including cell proliferation, receptor activation, and inhibition and contraction and proliferation of smooth muscle.

#### A. Role in Asthma and Chronic Obstructive Pulmonary Disease

PKCs have multiple signaling roles relevant to airway disease (Dempsey et al., 2007). PKC isoforms are involved in bronchoconstriction, as well as proliferation of airway smooth muscle cells. PKC activation by the phorbol ester phorbol 12,13-dibutyrate causes a slowly developing and sustained contraction of human airway smooth muscle that is blocked by the PKC inhibitor staurosporine (Rossetti et al., 1995). Several PKC isoforms are detectable in ASM (Webb et al., 1997; Donnelly et al., 1995). PKC-ζ plays a role in the proliferation of ASM cells in response to PDGF (Carlin et al., 2000). PKC activation in response to phorbol esters and acetylcholine results in uncoupling of β2-receptors in ASM, because diacylglycerol mediates phosphorylation of the β2-receptor and the stimulatory G-protein Gα (Grandordy et al., 1994). PKC is involved in the activation of murine Th2 cells through the phosphorylation and activation of voltage-gated calcium channels (CaV1.1-1.4) and PKC inhibition inhibits cytokine release from Th2 cells (Robert et al., 2014). PKC-δ is known to activate mTOR and suppression of PKC-δ by the inhibitor rottlerin inhibits PI3K-mTOR signaling after allergen exposure in a murine model of asthma (Choi et al., 2013). PKC-δ is involved in the activation of mast cells via IgE-receptors and regulates the synthesis and secretion of cysteinyl-leukotrienes (Cho et al., 2004a,b). PKC-δ is involved in activation of T-cells and IL-2 secretion so its inhibition causes immunosuppression (Chand et al., 2012). PKC-δ and PKC-ζ are involved in eosinophil migration (Langlois et al., 2009). Allergen challenge in patients with asthma results in long-term activation of PKC-ζ in eosinophils (Evans et al., 1999). PKC is also involved in the mucus secretion response and expression of MUC5AC after exposure to human neutrophil elastase (Shao and Nadel, 2005).

#### B. Protein Kinase C Inhibitors

The multiple roles of PKC are bronchoconstriction, inflammatory cell activation, mucus secretion, and remodeling and suggest that PKC inhibitors may have therapeutic potential in the management of asthma and COPD. Nonselective inhibitors, such as staurosporine, are too toxic for human administration, but several isoform-selective PKC inhibitors have been developed and entered clinical trials in various diseases, including
cancer, cardiovascular disease, diabetes, transplantation, and neuropsychiatric diseases (Mochly-Rosen et al., 2012). Most clinical trials have been negative and there have been several unexpected adverse effects. No clinical trials have been reported in asthma and COPD, and there are no PKC drugs currently approved for human therapy.

**XII. Other Kinase Targets**

Several other kinases may be potential therapeutic targets because they are involved in chronic inflammation or have effects on ASM.

**A. Rho-Associated Protein Kinase Inhibition**

Rho-associated protein kinases (ROCK1 and ROCK2) are Ser/Thr kinases that are mainly involved in regulating cytoskeletal function and thus the shape and movement of cells (Riento and Ridley, 2003). ROCK is therefore important in migration of inflammatory cells to sites of inflammation. ROCK1 is the major downstream target of the small GTPase RhoA. ROCK1 and ROCK2 phosphorylate actin and result in myosin light chain phosphorylation, resulting in contraction and increased contractility of smooth muscle. Thus ROCK plays a key role in determining contractility in bovine ASM (Gosens et al., 2004). ROCKs are thought to mediate some of the pleiotropic effects of statins.

The ROCK inhibitor Y-27632 administered by inhalation inhibits eosinophilic inflammation, AHR, and airway remodeling after chronic allergen exposure in sensitized guinea pigs (Schaafsma et al., 2008a; Possa et al., 2012; Righetti et al., 2014). This drug inhibits AHR, bronchoconstriction, and eosinophilic inflammation in a mouse model of acute allergic inflammation (Henry et al., 2005). Another ROCK inhibitor, fasudil, reduces airway inflammation and AHR induced by ozone exposure in mice (Kasahara et al., 2015). There is growing evidence that ROCK inhibitors inhibit fibrosis and so may reduce airway remodeling in airway diseases (Knipe et al., 2015). This suggests that ROCK inhibitors might be useful as bronchodilators and anti-inflammatory and antifibrotic therapy, but clinical development has been slow (Fernandes et al., 2007; Schaafsma et al., 2008b). Y-27632 [(R)-(+)−trans-4-(1-amo-noethyl)-N-(4-pyridyl)cyclohexanecarboxamide dihydrochloride] is not very selective for ROCK and may inhibit other kinases. It has not been developed clinically because of toxicity. Fasudil is a more selective ROCK inhibitor that has been approved in Japan for the treatment of cerebral vasospasm. There are no reported clinical studies in asthma or COPD. It is possible that there may be a positive interaction between a ROCK inhibitor and existing bronchodilator therapy, but there are no studies exploring this.

**B. Interleukin-1 Receptor-Associated Kinase Inhibition**

IL-1 receptor-associated kinases (IRAKs) are important enzymes in the signaling pathways from IL-1 and IL-18 receptors and from TLRs and are therefore upstream of NF-κB and MAPK activation. Of the four IRAKs identified, IRAK-4 appears to be the most important in inflammation and is therefore a potential target for anti-inflammatory therapy (Wang et al., 2009). In humans, mutations in IRAK-4 have been associated with susceptibility to bacterial infections. Small molecule IRAK-4 inhibitors are in clinical development and have been facilitated by the discovery of the crystal structure of IRAK-4 (Chaudhary et al., 2015). A naturally occurring compound lancemaside C inhibits macrophage activation induced by LPS via inhibition of IRAK-4 (Joh and Kim, 2010). An IRAK-4 inhibitor is effective in suppressing release of chemokines form airway epithelial cells of patients with COPD in vitro (Fenwick et al., 2013). To date no clinical studies with IRAK-4 inhibitors have been reported.

**C. Src Family Kinase Inhibition**

Proto-oncogene tyrosine protein kinase Src is a non-receptor tyrosine kinase linked to cell proliferation and malignancy but also involved in smooth muscle function and inflammation and may be activated by growth factors, adhesion molecules, and oxidative stress (Roskoski, 2015). Src family kinases play a key role in sensitizing airway smooth muscle and may play a role in AHR via activation of ROCK and is reversed by the Src inhibitor PP2 (Shaita et al., 2015). TNF-α disrupts airway epithelial barrier function and this is mediated via Src activation and prevented by a Src inhibitor SU6656 [2,3-dihydro-N,N-dimethyl-2-oxo-3-[(4,5,6,7-tetrahydro-1H-indol-2-yl)methylene]-1H-indole-5-sulfonamide] (Hardyman et al., 2013). The Src inhibitor PP2 and siRNA knockdown of Src prevents rhinovirus-induced release of CXCL8 from human airway epithelial cells (Bentley et al., 2007). Analysis of an asthma protein interactome suggests that Src plays a central role and may be a good target to inhibit (Randhawa and Bagler, 2012). Several Src inhibitors, such as dasatinib, have been developed for the treatment of hematologic malignancies but inhibit other kinases and have frequent side effects and have not been tested in airway disease.

**D. Hematopoietic Cell Kinase Inhibition**

Hematopoietic cell kinase (Hck), a Src family kinase, is a myeloid-specific nonreceptor tyrosine kinase involved in neutrophil production, adhesion and cytokine production. Hck overexpression in mice induces chronic neutrophilic inflammation and emphysema (Ernst, 2002). Hck is involved in neutrophil and myeloid cell recruitment into the lung, because Hck knockout mice fail to recruit these cells in response to TNF-α and LPS.
There is increased expression of Hck in circulating neutrophils from patients with COPD (Yanagisawa et al., 2009), suggesting that this kinase may be a good target for inhibition. Several drugs that inhibit Hck, including PP2 and dasatinib, are available, and new drugs are in development (Poh et al., 2015). Inhaled drugs that have inhibitory effects on Hck are also in clinical development for the treatment of severe asthma and COPD (Norman, 2014a; Onions et al., 2016).

E. Bruton's Tyrosine Kinase

Bruton's tyrosine kinase (BTK) plays a critical role in B-lymphocyte development and in mast cell activation through high-affinity IgE receptors (Crofford et al., 2016). Mutations of BTK are associated with X-linked agammaglobulinemia, which causes immunodeficiency. Selective BTK inhibitors, such as ibrutinib, have been developed for treatment of B cell malignancies, but so far have not been tested in airway disease (Foluso et al., 2016).

XIII. Future Developments

Although many hundreds of kinases have been implicated in chronic inflammatory diseases, relatively few have been successfully targeted therapeutically. Major problems involve specificity and adverse effects, often due to off-target effects and loss of efficacy over time. Although over 30 kinase inhibitors have been approved for the treatment of various forms of malignancy, relatively few are approved for the treatment of chronic inflammatory diseases and none so far for asthma and COPD therapy (Wu et al., 2015c).

A. Improving Selectivity and Safety

Most kinase inhibitors target the ATP-binding pocket, where they compete with ATP in high concentrations, and so need to be of at least nanomolar potency to be effective. Because ATP binding is common to all kinases, it is difficult to develop kinases with specificity for a particular kinase because of the great similarity in binding site between different kinases. The off-target effects often account for sometimes unexpected adverse effects that have severely limited the clinical development of these drugs. Although this is relatively less important for treatment of life-threatening malignancies, it is unacceptable in long-term treatment of chronic inflammatory diseases, such as asthma and COPD. Understanding the cocrystal structure of the ligand-ATP-binding site of particular kinases may aid the discovery of more selective inhibitors (Norman et al., 2012b). For example, this approach has been used to develop selective inhibitors of FGFR tyrosine kinase (Norman et al., 2012a). Targeting allosteric binding sites that lie outside the highly conserved ATP-binding site offers hope for greater potency and specificity and may also protect against the development of drug resistance by mutation of the ATP-binding site (Wu et al., 2015b). Binding may be to an allosteric site close or distant from the ATP-binding pocket. Over 10 allosteric kinase inhibitors are now in clinical development.

<table>
<thead>
<tr>
<th>Kinase</th>
<th>Inhibitors</th>
<th>Development Stage</th>
<th>Disease Indication</th>
<th>Comments</th>
</tr>
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<tr>
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<td>Phase 27</td>
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<td>Discontinued</td>
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<td>Oral</td>
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<td>B cell malignancy</td>
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<td>Cerebral vasospasm</td>
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</table>

ASK, apoptosis signal-regulatory kinase; EGFR, epidermal growth factor receptor; ERK, extracellular regulating kinase; IKK, inhibitor of kB kinase; JAK, Janus-activated kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappa B; PDGFR, platelet-derived growth factor receptor; PI3K, phosphoinositide-3-kinase; ROCK, Rho-associated protein kinase; RTK, receptor tyrosine kinase; Syk, spleen tyrosine kinase.
and some may be combined with conventional inhibitors of the same kinase.

Another way to limit side effects is to deliver kinase inhibitors topically and, in the case of airway disease, by inhalation, which should greatly reduce systemic exposure. This requires the development of drugs with high potency that have low oral bioavailability and topical activity. It has so far proven difficult to develop such drugs, but inhaled p38 MAPK inhibitors are currently in clinical development for severe asthma and COPD (Millan, 2011; Norman, 2015). Inhaled PI3Kγ/δ inhibitors are also in development for severe asthma and COPD because they may overcome the corticosteroids resistance in these diseases (Norman, 2014a; Onions et al., 2016).

B. Targeting Several Kinases

Inhibiting more than one kinase may increase efficacy because there may be additive or synergistic effects seen by inhibiting more than one kinase. In addition, it may decrease the risks of developing resistance by blocking pathways that may reduce the effect of monokinase inhibition (Morphy, 2010). Several multitargeted kinase inhibitors have been developed for the treatment of malignancies (Garuti et al., 2015). Sorafenib targets several tyrosine kinases (VEGFR, PDGFR, c-Raf, c-Kit), sunitinib (VEGFR, PDGFR), vandetanib (VEGFR, EGFR, RET), and pazopanib (VEGFR, PDGFR, c-Kit). These drugs are indicated for advanced malignancies, and adverse effects are common. Nintedanib inhibits several growth factor receptors (VEGFR, PDGFR, FGFR) and is approved for the treatment of idiopathic pulmonary fibrosis but has significant side effects (Keating, 2015). There is a case for the development of inhaled multitargeted kinase inhibitors for severe airway disease. Several narrow spectrum kinase inhibitors suitable for inhaled delivery have been developed for the treatment of corticosteroid-resistant inflammation in severe asthma and COPD (Onions et al., 2016). These drugs inhibit p38α, p38y and Hck and are more effective than p38α inhibitors in suppressing the release of inflammatory cytokines from macrophages in vitro and in treating inflammation in mice exposed to cigarette smoke, which is resistant to corticosteroids.

C. Overcoming Kinase Inhibitor Resistance

A major limitation to the use of kinase inhibitors in the treatment of malignancy is mutation of the ATP binding site, which overcomes the inhibitory effects of kinases. This is addressed by the development of multitargeted kinase inhibitors and allosteric kinase inhibitors, which make development of resistance less likely. Covalent kinase inhibitors are another approach, and irreversible inhibitors are now in clinical trials for malignant disease. Loss of effect of kinase incubators is also seen in the treatment of chronic inflammatory disease, such as rheumatoid arthritis and inflammatory bowel disease, and tends to develop over several weeks of treatment (Barouch-Bentov and Sauer, 2011). This may arise as compensatory pathways are upregulated to circumvent the pathway blocked by the kinase inhibitor and may be overcome by also blocking the compensatory pathway, for example with narrow spectrum kinase inhibitors (Onions et al., 2016).

D. Conclusions

Although this review highlighted the involvement of many different kinases in both asthma and COPD, so far no kinase inhibitors have been approved for clinical use and have not even yet reached Phase 3 studies. Table 1 summarizes the clinical status of many of the drugs that are discussed in the review. Many preclinical studies and early clinical trials are underway, so hopefully the field will advance. A major challenge has been the development of potent inhaled kinase inhibitors to avoid systemic exposure and the lack of such compounds has slowed development in this area.

In view of the pressing unmet need for new treatments in severe asthma and COPD, it is hoped that there will be significant investment for the pharmaceutical industry in this area in the future.

References


Kinase Inhibitors in Airway Disease


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Ziegelbauer K, Gantner F, Lukacs NW, Berlin A, Fuchikami K, Niki T, Sakai K, Inbe Y a m a m o t o N , T a k e s h i t a K , S h i c h i j o M , K o k u b o T , S a t o M , N a k a s h i m a K , I s h i m o r i M , N a g a i H , L i Y F , and Y u r a T , et al. (2003) The orally available spleen tyrosine kinase inhibitor 2-[7-(3,4-dimethoxyphenyl)-imidazo[1,2-c]pyrimidin-5-ylamino]nicotinamide dihydrochloride (BAY 61-3606) blocks antigen-induced airway inflammation in rodents. J Pharmacol Exp Ther 306: 1174–1181.

Ziegelbauer K, Gantner F, Lukacs NW, Berlin A, Fuchikami K, Niki T, Sakai K, Inbe Y a m a m o t o N , T a k e s h i t a K , S h i c h i j o M , K o k u b o T , S a t o M , N a k a s h i m a K , I s h i m o r i M , N a g a i H , L i Y F , and Y u r a T , et al. (2003) The orally available spleen tyrosine kinase inhibitor 2-[7-(3,4-dimethoxyphenyl)-imidazo[1,2-c]pyrimidin-5-ylamino]nicotinamide dihydrochloride (BAY 61-3606) blocks antigen-induced airway inflammation in rodents. J Pharmacol Exp Ther 306: 1174–1181.