

Airway Smooth Muscle Cell as Therapeutic Target of Inflammation

Chi-Ming Hai*

Department of Molecular Pharmacology, Physiology & Biotechnology, Brown University, Providence, Rhode Island 02912, USA

Abstract: Airway inflammation is an outcome of complex interactions of multiple cell types in an inflammatory network. In recent years, it has become clear that a single target approach is unlikely to be effective for the treatment of inflammatory airway diseases such as asthma. This recognition suggests an alternative approach of targeting multiple cell types and/or mediators. Airway smooth muscle (ASM) cells are unique in serving the dual function of bronchoconstriction and inflammation in the airway system. ASM cells respond to a large array of external stimuli such as acetylcholine, bradykinin, inflammatory cytokines, and cyclic stretch with the expression of inflammatory mediators such as cytokines and cyclooxygenase products. Ca^{2+} influx through voltage-gated and transient receptor potential channels are important mechanisms of Ca^{2+} -dependent transcription in ASM cells. Calcineurin and Ca^{2+} , calmodulin-dependent kinase (CaMK) are Ca^{2+} -sensitive enzymes that regulate the activation of the two transcription factors, nuclear factor of activated T-cells (NFAT) and cyclic AMP response element binding protein (CREB). Erk1/2 and p38 mitogen-activated protein kinases are signaling enzymes that couple receptor activation to gene transcription by phosphorylating CREB and stabilizing mRNA against de-adenylation. CREB is a unique transcription factor that is phosphorylated by both CaMK II and Erk1/2 MAPK. Nuclear factor B (NF B) appears to be a universal transcription factor that regulates the transcription of almost all inflammatory genes. Detailed understanding of the cellular components and interactions in the inflammatory network of the airway system may lead to rational targeting of multiple cells and mediators in the treatment of airway inflammation.

Keywords: Airway, Asthma, Cytokine, Inflammation, Lung, Network, Stretch, Ventilation.

1. INTRODUCTION

Airway inflammation is a central problem in allergic asthma and ventilation-induced lung injury. Airway inflammation is an outcome of complex interactions of multiple cell types in an inflammatory network. These cells include epithelial cells, inflammatory cells, airway smooth muscle cells, and others. Most cells in an inflammatory network function both as sources and effectors of a large array of inflammatory mediators. Such iterative interactions result in the amplification of the overall inflammatory response and the recruitment of additional inflammatory cells to the airway system [49, 158]. Fig. (1) shows the interactions among epithelial cells, inflammatory cells, and airway smooth muscle cells in the regulation of inflammatory mediator release in the airway system. The array of inflammatory mediators in Fig. (1) includes cytokines, chemokines, and prostanoids [17, 19]. Understanding the cellular components and the interconnections of the inflammatory network in the airway system may allow rational targeting of multiple cell types and/or molecules in the control of airway inflammation [3]. Airway smooth muscle cells in culture are capable of secreting pro-inflammatory cytokines, chemokines, growth factors, extracellular matrix proteins, and cell adhesion receptors in response to inflammatory cytokines [61, 90]. Recent studies suggest that most extracellular stimuli that elicit airway smooth muscle contraction, such as bradykinin,

acetylcholine, histamine, and cyclic stretch, are also capable of stimulating inflammatory cytokine expression in airway smooth muscle cells. This review provides an integrative overview of the molecular mechanisms by which airway smooth muscle cells perform their function as inflammatory cells in the airway system, and discusses the possibility of targeting airway smooth muscle cells in the control of airway inflammation.

2. EXTRACELLULAR STIMULI THAT INDUCE INFLAMMATORY MEDIATOR RELEASE BY AIRWAY SMOOTH MUSCLE CELLS

2.1 Inflammatory Cytokines

Airway smooth muscle cells in culture secrete cytokines and chemokines in response to inflammatory cytokines [61, 64, 148]. In an inflammatory network, inflammatory cytokines serve as autocrines and/or paracrines for the stimulation of inflammatory mediator release from multiple cell types including airway smooth muscle cells as shown in Fig. (1). Table 1 lists the extracellular stimuli that have been reported to stimulate inflammatory mediator release from airway smooth muscle cells. Careful analysis of the data shown in Table 1 suggests several interesting characteristics.

First, a search of Table 1 for the inflammatory stimuli that induce the release of a given cytokine reveals the existence of redundant receptor systems. For example, the interleukins (IL's) IL-1, IL-4, IL-9, IL-13, and TNF- are all capable of stimulating the expression and/or release of eotaxin by airway smooth muscle cells (Table 1). Similarly, IL-1, IL-1, IL-17, TGF-, and TNF- are all capable of

*Address correspondence to this author at the Department of Molecular Pharmacology, Physiology & Biotechnology, Brown University, Box G-B3, 171 Meeting Street, Providence, Rhode Island 02912, USA; Tel: (401) 863-3288; Fax: (401) 863-1222; E-mail: Chi-Ming_Hai@brown.edu

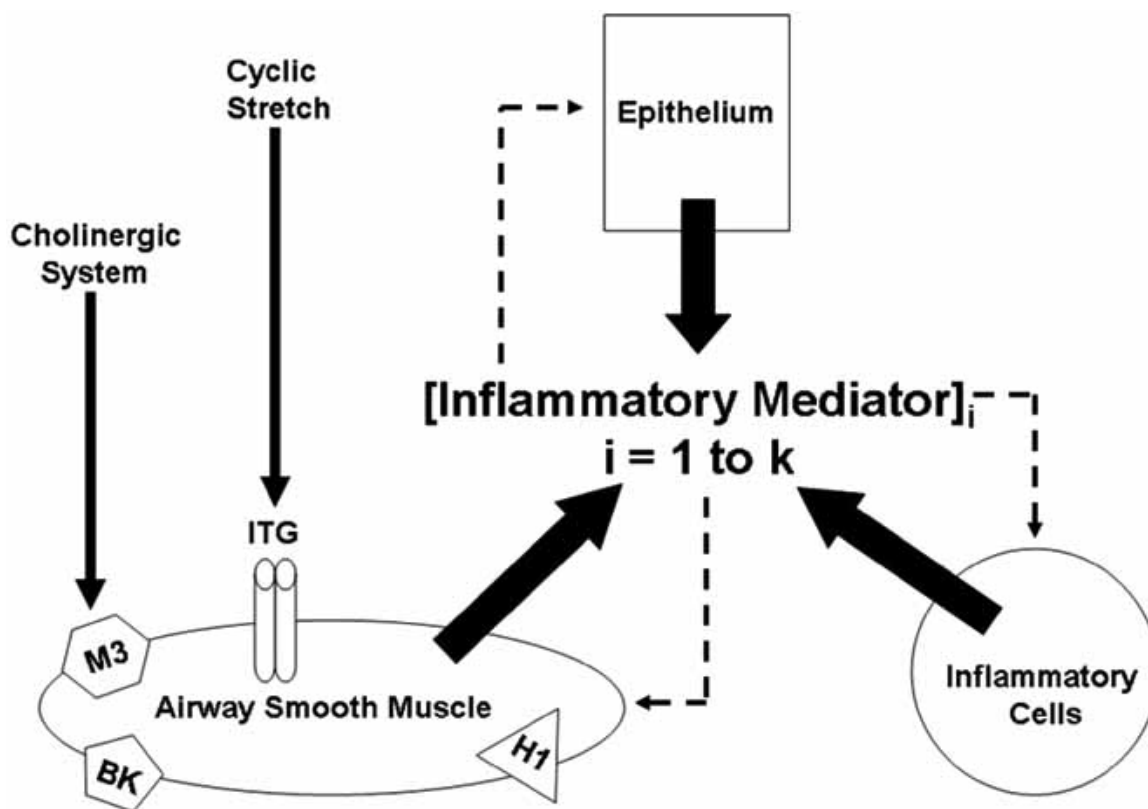


Fig. (1). A simplified model of inflammatory network in the airway system showing interactions among epithelial cells, inflammatory cells, and airway smooth muscle cells, with a special emphasis on the inflammatory function of airway smooth muscle cells. $[\text{Inflammatory Mediator}]_i$, $i = 1$ to k represents the array of inflammatory mediators that are both products and stimuli in the inflammatory network. Neuronal and non-neuronal cholinergic systems stimulate the release of inflammatory mediators by airway smooth muscle cells by activating m3 muscarinic receptors (M3). Bradykinin stimulates the release of inflammatory mediators from airway smooth muscle cells by activating the bradykinin receptor (BK). Cyclic stretch stimulates the release of inflammatory mediators from airway smooth muscle cells by mechanosensitive activation of integrin receptors (ITG).

stimulating the expression and/or release of IL-8 from airway smooth muscle cells (Table 1). One therapeutic implication of having redundant receptor systems for the release of the same cytokine is that targeting a single cytokine receptor system is unlikely to be effective for the treatment of airway inflammation.

Second, a search of Table 1 for identity between stimulus and product in Table 1 reveals little evidence of direct positive feedback between stimulus and product for airway smooth muscle; that is, when the product is the same as the stimulus. The apparent lack of direct positive feedback for airway smooth muscle suggests that airway smooth muscle cells alone are not capable of amplifying an inflammatory response in the absence of other inflammatory cells in the vicinity. However, the possibility of indirect positive feedbacks within the inflammatory network in the airway system cannot be excluded. For example, airway smooth muscle cells may respond to a stimulus X with the production of a product Y, which may in turn stimulates the production of X from another cell in the vicinity, resulting in the amplification of both X and Y. Therefore, recruitment of inflammatory cells to the vicinity of airway smooth muscle cells may be a necessary step for airway smooth muscle cells to participate in the amplification of airway inflammation.

Cyclooxygenase (COX) products have been implicated in the inflammatory process in asthma [19, 141]. However, COX-2 products are diverse in their actions as both pro- and anti-inflammatory mediators in airway inflammation [126]. For example, prostaglandin D_2 is a pro-inflammatory molecule. In contrast, prostaglandin E_2 is an anti-inflammatory molecule. A search of Table 1 for stimuli that induce cyclooxygenase-2 (COX-2) expression reveals parallel up-regulation of IL-6 and IL-8 in airway smooth muscle cells. For example, both bradykinin and IL-1 induce parallel up-regulation of COX-2, IL-6, and IL-8 in airway smooth muscle cells, suggesting coordinated regulation of the expression of these three molecules. Using COX-2 inhibitors, Pang and Knox [121] have demonstrated the role of cyclooxygenase products in mediating bradykinin-stimulated IL-8 production in cultured airway smooth muscle cells. Similarly, using cells over-expressing COX-2, Dalwalwadi *et al.* [28] have demonstrated the dependence of IL-6 expression on COX-2 expression in cells. These findings together suggest the critical role of COX-2 in the regulation of IL-6 and IL-8 expression in airway smooth muscle cells.

Leukotrienes are important mediators of airway inflammation in asthma [135]. Accordingly, receptor antagonists have been developed to target leukotriene-

Table 1. Extracellular Stimuli That Induce the Release of Inflammatory Mediators by Airway Smooth Muscle Cells

Cultured Airway Smooth Muscle Cells		
Stimulus	Product	References
IFN-	CXCL10	[58]
IL-1	IL-8	[162]
IL-1	CCL2, COX-2, IL-6, IL-8,	[22, 50, 71, 87, 89, 110,
	Eotaxin, RANTES	[128, 132]
IL-4	Eotaxin	[62, 108, 131, 174]
IL-9	Eotaxin	[47]
IL-13	CCL2, CCL26, CCL27, Eotaxin	[62, 71, 108, 131, 151,
		174]
IL-17	IL-8	[140, 157, 169]
TGF-	IL-8, IL-11, COX-2	[34, 38, 71]
TNF-	IL-6, IL-8, CXCL10, Eotaxin,	[8, 10, 22, 50, 58, 75, 104,
	RANTES	112, 162]
IFN- + TNF-	Fractalkine	[150]
IL-1 + IFN- + TNF-	COX-2, IL-1, IL-6, IL-8	[59, 147]
Bradykinin	COX-2, IL-6, IL-8, RANTES	[67, 110, 121, 122, 125,
		132, 136, 173]
Cyclic Stretch	IL-8	[84]
<i>Intact Airway Smooth Muscle</i>		
IL-5	IL-1	[53]
IL-1 + TNF-	IL-1, IL-6, IL-8, RANTES	[52]
TNF-	COX-2	[12]
Carbachol	CCL2, COX-2, IL-6, IL-8,	[79]
Cyclic Stretch	CCL2, COX-2, IL-6, IL-8	[79]

induced eosinophilia and airway obstruction in asthmatic patients [88]. In theory, airway smooth muscle cells may be capable of producing leukotrienes from the conversion of arachidonic acid by lipoxygenases. However, relatively little is known about the function of lipoxygenase pathways in airway smooth muscle cells.

Growth factors such as neurotrophins have profound effects on various immune cells involved in airway inflammation [111]. A recent finding indicates that airway smooth muscle cells express nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 constitutively and in response to cytokine stimulation [81]. These findings suggest that airway smooth muscle cells may participate in the interaction between the neuronal and immune cells in the airway system.

As shown in Table 1, much of the literature on the secretory function of airway smooth muscle cells is based on data collected from cultured cell experiments. Smooth muscle cells are capable of undergoing significant

phenotypic modulation ranging from a highly synthetic/proliferative phenotype to a highly contractile/differentiated phenotype depending on the chemical and mechanical environment [116]. Therefore, data derived from cultured cell studies should be interpreted with caution. It is important to perform experiments on intact airway smooth muscle to determine to what extent inflammatory genes are expressed in intact differentiated airway smooth muscle.

2.2 Bradykinin

Bradykinin is known to mediate inflammation in multiple organ systems including the airway system [91]. Bradykinin is a nine-amino-acid peptide produced from proteolysis of high and low molecular-weight kininogens in the plasma [1]. Inflammatory stimuli activate tissue kallikrein, the major kininogenase in the airway system, thereby stimulating the production of bradykinins that

causes bronchoconstriction and airway hyperresponsiveness [1]. Bradykinin B2 receptor antagonists and indomethacin are effective in inhibiting bradykinin-stimulated contraction, suggesting the involvement of bradykinin B2 receptor and COX products in bradykinin-induced airway smooth muscle contraction [107]. Bradykinin stimulates the expression of IL-6, IL-8, COX-2, and regulated on activation, normal T cell expressed and secreted (RANTES) in cultured airway smooth muscle cells (Table 1). Bradykinin-stimulated release of IL-6 and IL-8 from airway smooth muscle cells appears to be mediated by COX-2 products [121]. These findings are significant since IL-6 is an important mediator of allergic asthma because of its role in the expansion of T-helper 2 cells [30]. Similarly, IL-8 is a potent chemokine for the recruitment of neutrophils to the airway system in inflammatory lung diseases [129]. Despite the often coordinated expression of COX-2, IL-6 and IL-8, transcriptional controls of the expression of these three molecules are different. The cyclic AMP-response element regulates bradykinin-stimulated COX-2 transcription [110]. In contrast, prostanoid-dependent activation of AP-1 and NF-IL-6 regulates bradykinin-stimulated IL-8 transcription [173]. By stimulating the release of IL-6 and IL-8 from airway smooth muscle cells, bradykinin may engage airway smooth muscle cells in the inflammatory response in the airway system. Given the dual roles of bradykinin as contractile as well as inflammatory stimuli for airway smooth muscle cells, bradykinin may be a useful target in the treatment of airway inflammation and bronchoconstriction in inflammatory airway diseases such as asthma.

2.3 Acetylcholine

Cholinergic neuronal and non-neuronal sources both contribute to the release of acetylcholine in the airway system [13, 139, 163]. Acetylcholine stimulates airway smooth muscle contraction mostly by activating m3 muscarinic receptors [15]. In addition, m2 muscarinic receptors on cholinergic neurons function as effectors in a negative feedback loop to prevent the excessive neuronal release of acetylcholine. Accordingly, inflammatory cell-mediated dysfunction of neuronal m2 muscarinic receptors has been proposed as a mechanism of excessive release of acetylcholine from cholinergic nerves in asthma [15, 26]. Recent clinical studies indicate the effectiveness of anti-muscarinic cholinergic drugs in the treatment of asthma; however, the pharmacologic effects have often been attributed to the control of airway smooth muscle contraction [15, 78]. Recent studies suggest the intriguing possibility that muscarinic cholinergic receptor activation may also stimulate mitogenesis and inflammatory gene expression in airway smooth muscle cells [44, 79]. For example, m3 muscarinic receptor agonists potentiate growth factor-mediated proliferation of cultured airway smooth muscle cells [43]. Accordingly, the muscarinic receptor antagonist, tiotropium bromide, inhibits allergen-induced airway smooth muscle proliferation [42]. It is noteworthy that m3 muscarinic cholinergic receptors are coupled to the G_q subunit [21], the same subunit that mediates bradykinin-stimulated IL-6 secretion by cultured airway smooth muscle cells [66, 136]. Recently, Kanefsky *et al.*

[79] have reported that cholinergic receptor activation stimulates the expression of IL-6, IL-8, COX-2 and other inflammatory cytokines and chemokines in intact airway smooth muscle. This finding is consistent with the observed pro-inflammatory effect of muscarinic cholinergic stimulation on other cell types. For example, muscarinic cholinergic receptor activation augments lymphocyte-mediated cytotoxicity [149], stimulates release of chemotactic activity by alveolar macrophages [144], and stimulates the release of neutrophil and monocyte chemotactic activity by bronchial epithelial cells [82]. Therefore, these findings together suggest the intriguing possibility that parasympathetic neuronal and non-neuronal cholinergic systems in the airway system may stimulate the release of inflammatory mediators from airway smooth muscle cells in addition to their stimulatory effects on bronchoconstriction.

2.4 Histamine

Histamine performs the dual function of stimulating the contraction of airway smooth muscle cells and the secretion of proinflammatory cytokines and chemokines by inflammatory cells [6, 98, 100, 118, 154]. For example, H1 receptor agonists stimulate IL-6 production from human lung macrophages [155]. Similarly, histamine exerts pro-inflammatory effects on keratinocytes via H1 receptors [41]. Accordingly, the H1 receptor antagonist desloratadine has anti-inflammatory effects on allergic rhinitis [4]. Similarly, the histamine H1 receptor antagonist olopatadine completely inhibited histamine-induced production of IL-6 and IL-8 in keratinocytes [102]. In mice, treatment with histamine receptor antagonist prior to and during sensitization is effective in suppressing allergen-induced Th2 responses as well as development of eosinophilic airway inflammation [16]. Using histidine decarboxylase gene-targeted knockout (HDC-KO) mice lacking histamine, Kozma *et al.* [83] have shown that ovalbumin-sensitized and challenged HDC-KO mice exhibit reduced airway hyperresponsiveness and lung inflammation in comparison with wild-type mice. The levels of IL-1, IL-1, IL-4, IL-5, IL-6, and IL- are also significantly lower in the HDC-KO mice in asthmatic late phase, indicating a significantly altered immune response to ovalbumin provocation and challenge. These findings highlight the importance of histamine H1 receptors in mediating the release of inflammatory cytokines and chemokines by inflammatory cells.

Histamine H1 receptors are known to mediate the stimulatory effect of histamine on airway smooth muscle contraction [73]. In addition, histamine up-regulates c-fos expression and stimulates the proliferation of cultured airway smooth muscle cells [120]. This finding is significant because c-fos is an early gene product that is involved in the inflammatory gene expression in human airway smooth muscle cells such as the stimulation of IL-6 release in response to pro-inflammatory cytokines [103]. The existence of histamine H1 receptors together with the ability of histamine to stimulate c-fos expression in airway smooth muscle cells suggest the intriguing possibility that histamine H1 receptors may also mediate inflammatory gene expression in airway smooth muscle cells.

2.5 Cyclic Stretch

Airway smooth muscle cells function in a mechanically active environment during breathing cycles. Stretching of the airway system is known to have both beneficial and deleterious effects. For example, deep inspirations are known to have bronchodilatory and bronchoprotective effects on normal airways [146]. At the same time, ventilation with high tidal volume is recognized as an important cause of ventilation-induced lung inflammation and injury [133, 156]. In extreme cases, mechanical ventilation can result in the release of lung-borne inflammatory mediators into the systemic circulation, thereby causing multiple system organ failure [55, 134]. In patients with acute lung injury, plasma levels of inflammatory cytokines such as IL-6 and IL-8 are associated with morbidity and mortality [127].

In perfused lungs [159] and anesthetized mice [165], hyperventilation stimulates the expression and release of inflammatory cytokines such as TNF- α and IL-6. In the rat lung, high tidal volume ventilation for as brief as 30 min stimulates significant up-regulation of inflammatory genes and transcription factors before any discernable lung injury as measured by mechanics or histology [24]. In rabbit lungs, even mechanical ventilation with a moderate tidal volume elicits the up-regulation of inflammatory gene expression [18]. A recent study on TNF- α knockout animals suggests that TNF- α mediates a substantial component of ventilation-induced lung inflammation [166]. These animal studies suggest that cyclic stretch is a potent stimulus of lung inflammation.

Multiple cell types in the airway system are capable of producing inflammatory mediators in response to cyclic stretch. For example, cultured alveolar epithelial cells, alveolar macrophages, and airway smooth muscle cells release inflammatory mediators in response to cyclic stretch [31, 56, 84, 115, 137]. Recently, Kanefsky *et al.* [79] have reported that intact airway smooth muscle responds to cyclic stretch with an up-regulation of inflammatory gene expression [79]. Interestingly, within the physiological range of tidal breathing [39], frequency of cyclic stretch has been found to be an important determinant of inflammatory gene expression in intact airway smooth muscle [79] and ventilator-induced lung injury in animals [23]. These findings suggest that both frequency and amplitude of cyclic stretch are important determinants of inflammatory gene expression in airway smooth muscle and possibly other cell types in the airway system. Cyclic stretch also stimulates the expression of inflammatory gene expression in vascular smooth muscle cells [172] and uterine smooth muscle cells [96]. These findings suggest that cyclic stretch-stimulated inflammatory gene expression may be a general property of all smooth muscle cells.

3. SIGNALING PATHWAYS LEADING TO INFLAMMATORY GENE TRANSCRIPTION

3.1 Ca²⁺ Influx Mechanisms

Stimulation of Ca²⁺ influx is an important mechanism of depolarization and receptor-mediated gene transcription in vascular smooth muscle cells [13, 161] and neurons [37]. L-type voltage-gated calcium channels, store-operated calcium

channels, and non-specific cation channels are major Ca²⁺ entry pathways involved in the regulation of Ca²⁺-dependent transcription in vascular smooth muscle cells [13, 113, 152]. For example, both depolarization-stimulation of voltage-gated calcium channels and thapsigargin-mediated activation of store-operated calcium channels stimulate the expression of cyclic AMP-response element (CRE)-containing genes in vascular smooth muscle cells [138]. Similarly, thapsigargin-mediated activation of store-operated calcium channels stimulates IL-6 secretion by cultured human airway smooth muscle cells [66]. Recently, voltage-gated calcium channels have been implicated in cyclic stretch-stimulated gene expression of IL-6, IL-8, COX-2, and other inflammatory cytokines in intact airway smooth muscle [79]. Together these findings suggest that Ca²⁺ influx pathways are important signaling mechanisms in the regulation of inflammatory gene expression in airway smooth muscle cells.

The discovery of transient receptor potential channels from cloning experiments has provided molecular identity to some cation channels such as store-operated calcium channels and non-specific cation channels [117]. The transient receptor potential C6, V4, and other related transient receptor potential channels have been identified in cultured airway smooth muscle cells [25, 72, 114]. Accordingly, transient receptor potential channels have been suggested as therapeutic targets in the control of airway smooth muscle contraction in asthma [45, 113]. Recently, the transient receptor potential channel TRPC3 has been shown to mediate TNF- α -stimulated Ca²⁺ influx in human airway smooth muscle cells [164]. This finding is potentially significant for understanding airway inflammation because TNF- α is an important stimulus for the release of inflammatory mediators by airway smooth muscle cells (Table 1). Calcium influx mechanisms have been suggested as therapeutic targets in controlling the inflammatory response of neutrophils [153]. Similarly, targeting calcium influx mechanisms in airway smooth muscle cells may be equally effective in controlling inflammatory gene expression in the airway system.

3.2 Ca²⁺-Sensitive Enzymes of Gene Transcription

Calcineurin and Ca²⁺,calmodulin-dependent kinases (CaMK's) are Ca²⁺-sensitive enzymes that regulate the activation of the two transcription factors, nuclear factor of activated T-cells (NFAT) and cyclic AMP response element binding protein (CREB), respectively [13]. Calcineurin is a Ca²⁺-sensitive phosphatase that is essential for the development and function of the immune system as well as other organ systems [99]. Therefore, calcineurin inhibitors such as cyclosporine A and FK506 have strong immunosuppressive effects as well as severe side effects on many organ systems [101]. Calcineurin inhibitors are useful immunosuppressants in organ transplantation. However, the severe toxic effects of calcineurin inhibitors on almost all organ systems will probably limit their usefulness in the treatment of airway inflammation.

CaMK's are Ca²⁺-dependent enzymes that catalyze the phosphorylation of the transcription factor CREB in smooth muscle [13, 74]. *In vitro* studies have demonstrated that CaMK II activation by autophosphorylation is sensitive to

the frequency of Ca^{2+} spikes [29], suggesting that CaMK II may function as a frequency decoder in Ca^{2+} pulse-regulated gene expression [33, 68]. Additional studies have further identified CaMK II as a frequency decoder of Ca^{2+} pulses in excitation-transcription coupling in neurons and cardiac muscle cells [11, 33, 171]. Cyclic stretch-stimulated inflammatory gene expression in airway smooth muscle is sensitive to oscillatory frequency and dependent on Ca^{2+} influx through voltage-gated calcium channels [79]. Therefore, integrin-mediated mechanosensitive Ca^{2+} entry [69] and CaMK II-mediated frequency decoding are possible mechanisms of cyclic stretch-stimulated gene expression in airway smooth muscle. CaMK has been implicated in oxidant stress-stimulated I B phosphorylation in T lymphocytes [65], platelet-activating factor-mediated priming of macrophages [27], and TNF- α -induced CD44 expression in human monocytic cells [105]. Given the essential role of calcineurin in immune cell development, CaMK may be an alternative Ca^{2+} -dependent enzyme of inflammatory gene expression that can be targeted in the treatment of airway inflammation.

3.3 Mitogen-Activated Protein Kinases (MAPKs) as Regulators of Inflammatory Gene Expression

Erk1/2 and p38 MAPKs are coupling kinases that transduce receptor activation to gene transcription in many cell types including airway smooth muscle cells [40, 59, 130]. MAPK is regulated by a three-module enzyme cascade consisting of MAPK, MAPK Kinase, and MAPK Kinase Kinase. The Raf/MEK/Erk1/2 MAPK cascade is activated by the small G-protein Ras [130]. For the p38 MAPK cascade, p38 MAPK can be activated by MKK3/6 which in turn can be activated by TGF- β -activated kinase 1 (TAK1) or apoptosis signal-regulating kinase 1 (ASK1). An important function of Erk1/2 MAPK signaling pathway is the phosphorylation of CREB [40]. An important function of the p38 MAPK signaling pathway is the stabilization of mRNA against de-adenylation after transcription [84, 143]. There is evidence of crosstalk between the Ca^{2+} and MAPK signaling pathways because Ca^{2+} and calmodulin modulate the progression of the Ras/Raf/MEK/Erk activation cascade [5]. Similarly, Ca^{2+} oscillations lower the effective Ca^{2+} threshold for the activation of the Ras/Erk/MAPK signaling cascade [86].

p38 MAPK plays an important regulatory role in inflammation [145]. For example, p38 $^{-/-}$ embryonic stem cells fail to produce IL-6 in response to IL-1 stimulation [7], indicating that p38 is essential for IL-1-mediated IL-6 production. In cultured airway smooth muscle cells, p38 MAPK has a recognized role in inflammatory cytokine-stimulated COX-2 expression [59, 89, 147]. In addition, both Erk1/2 and p38 MAPK have multiple effects on cultured airway smooth muscle cells including the stimulation of IL-8 expression by IL-17, bradykinin, and cyclic stretch [67, 84, 169], COX-2 expression by TNF- α [93], and eotaxin release by IL-4 and IL-13 [108, 131]. Altogether, these findings indicate that Erk1/2 and p38 MAPK are critical signaling enzymes in the regulation of inflammatory gene expression in airway smooth muscle cells.

In addition to its direct effects on inflammatory gene expression, Erk1/2 MAPK also regulates prostaglandin

synthesis by inducing the expression of cytosolic phospholipase A₂, an enzyme critical for releasing arachidonic acid from membrane phospholipid in airway smooth muscle cells [97]. Arachidonic acid is then converted to prostaglandins through COX-1 and COX-2 enzymes.

4. TRANSCRIPTION FACTORS IN INFLAMMATORY GENE EXPRESSION

A detailed discussion of the large number of transcription factors involved in the regulation of inflammatory gene expression in asthma [142] is beyond the scope of this review. Instead, this review focuses on the two transcription factors CREB and NF- κ B in the regulation of inflammatory gene expression in airway smooth muscle cells. CREB is significant for the regulation of gene expression in smooth muscle cells because it is a substrate of both CaMK II and Erk1/2 MAPK in the regulation of inflammatory gene transcription [13, 40, 130, 161, 167]. Similarly, nuclear factor κ B (NF- κ B) appears to be a universal transcription factor that regulates the transcription of a large number of inflammatory genes [92, 95].

CREB binds to the CRE, which is present at the promoter region of many inflammatory genes such as COX-2, IL-6, IL-8, plasminogen activator urokinase (PLAU), RANTES, and TNF- α [20, 32, 70, 94, 110, 160]. It remains unknown whether CRE is present at the promoter region of chemokine C-C motif ligand 2 (CCL2) gene, a gene that was up-regulated by cyclic stretch. However, 8-bromo-cAMP reverses the effects of COX-2 inhibition on CCL2 expression induced by TNF- α and IL-1 in hepatic stellate cells [35], suggesting that cAMP mediates the effect of prostaglandins on CCL2 expression. In contrast, 8-bromo-cAMP decreases CCL2 expression induced by IL-1 in human airway smooth muscle cells [168], suggesting that cAMP antagonizes CCL2 expression. Therefore, cAMP appears to regulate CCL2 expression although the exact role of cAMP remains unclear. In cultured airway smooth muscle cells, CRE activation is critical for bradykinin-stimulated COX-2 transcription [110]. These findings together suggest that CREB phosphorylation is involved in cyclic stretch-stimulated expression of COX-2, IL-6, IL-8, PLAU, and possibly CCL2 in intact airway smooth muscle.

Nuclear factor κ B (NF- κ B) is a group of structurally related transcriptional proteins that control the transcription of a large number of genes involved in immunity and inflammation [92, 95]. In cultured airway smooth muscle cells, NF- κ B has been shown to mediate inflammatory cytokine-stimulated COX-2 transcription [147] and bradykinin-stimulated IL-8 transcription [173].

Fig. (2). summarizes the regulatory mechanisms of inflammatory gene expression in airway smooth muscle cells as discussed in this review.

5. AIRWAY SMOOTH CELLS AS THERAPEUTIC TARGETS OF AIRWAY INFLAMMATION

Airway smooth muscle cells are unique in serving the dual functions of airway constriction and inflammation. These two functions of airway smooth muscle appear to be intertwined as many contractile stimuli such as cholinergic

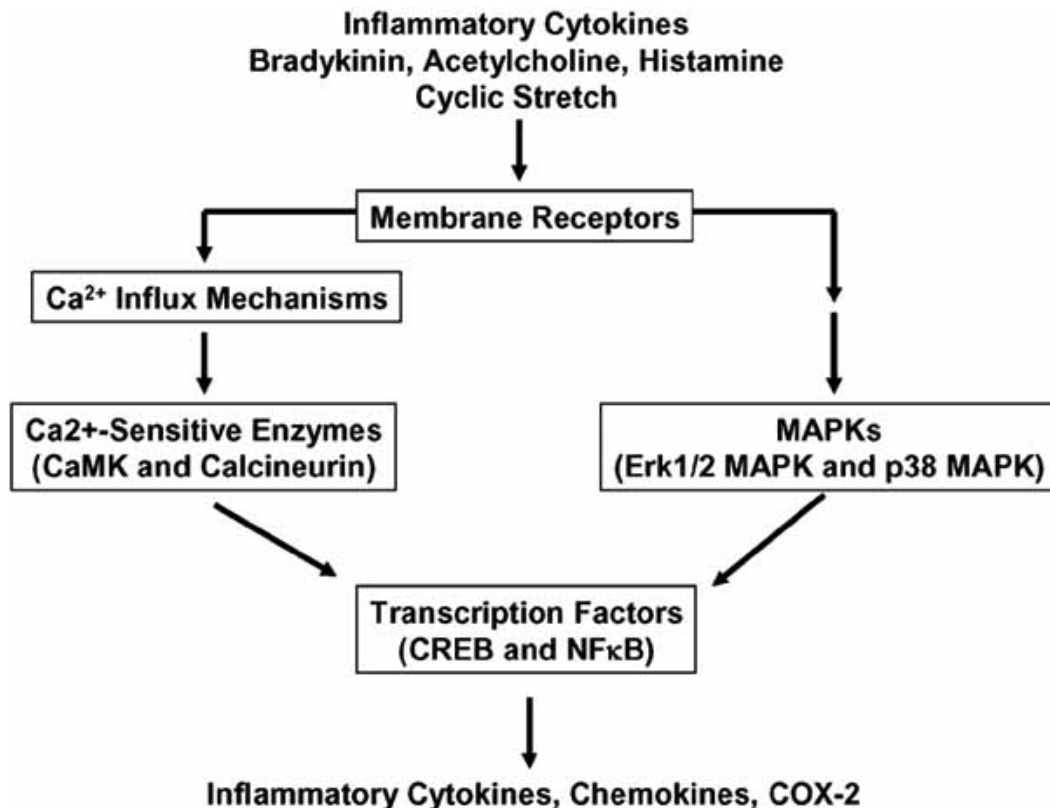


Fig. (2). Regulatory mechanisms of inflammatory gene expression in airway smooth muscle. This diagram summarizes the mechanisms presented in this review. Extracellular stimuli such as inflammatory cytokines, bradykinin, acetylcholine, histamine, cyclic stretch activate membrane receptors, thereby activating calcium channels, Ca²⁺-sensitive enzymes, and mitogen-activated protein kinases (MAPKs). These signaling enzymes in turn activate the transcription factors, cyclic AMP-response element-binding protein (CREB) and nuclear factor- B (NF B), thereby stimulating the expression of inflammatory cytokines, chemokines, and cyclooxygenase-2 (COX-2) in airway smooth muscle.

stimulation and cyclic stretch also stimulate inflammatory gene expression in airway smooth muscle cells. Review of the literature suggests the hypothesis that stimulation of airway smooth muscle contractility may always be accompanied by inflammatory gene expression. This hypothesis suggests that controlling airway smooth muscle contractility may also be effective for the treatment of airway inflammation.

Airway smooth muscle is also unique in its ability to respond to a large array of external stimuli such as acetylcholine, histamine, bradykinin, inflammatory cytokines, and cyclic stretch with the production of inflammatory cytokines. Some receptors such as bradykinin B2 have already been targeted for the treatment of airway inflammation in perennial rhinitis and asthma [60]. However, the intriguing possibility that muscarinic acetylcholine receptors may be pro-inflammatory in the airway system remains to be further tested.

Corticosteroids and 2-agonists currently constitute the first line of drug therapy in asthma [54]. Corticosteroids are well known for their anti-inflammatory effects on inflammatory cells. 2-agonists are well known for their bronchodilatory effects on airway smooth muscle cells. However, recent findings suggest that corticosteroids may also inhibit the inflammatory function of structural cells of the airway system including airway smooth muscle cell

[119]. For example, dexamethasone inhibits IL-1 /TNF- -stimulated inflammatory gene expression in airway smooth muscle cells [52]. Similarly, recent findings suggest that 2-agonists may also have anti-inflammatory effects on both resident cells and circulating inflammatory cells in the airway system [57]. For example, 2-agonists and corticosteroids inhibit TNF- -stimulated release of eotaxin, IL-6, IL-8, and RANTES by human airway smooth muscle cells [9, 22, 109, 123, 124]. These findings suggest that airway smooth muscle cells should be considered as targets of the anti-inflammatory effects of corticosteroids and 2-agonists. High-affinity IgE receptors have recently been identified on the surface of airway smooth muscle cells [46, 48]. Furthermore, activation of these receptors stimulates the release of cytokines and chemokines by airway smooth muscle cells. These findings provide additional support for the emerging view that airway smooth muscle cells function as inflammatory cells in the airway system.

Deep inspirations are known to have bronchodilatory and bronchoprotective effects on normal airways [146]. However, ventilation with high tidal volume is an important cause of ventilation-induced lung inflammation and injury [133, 156]. In the airway system, airway smooth muscle cells may function as biomechanical sensors in the modulation of airway inflammation in response to changes in tidal volume and frequency of breathing cycles. Given the beneficial and

deleterious effects of lung ventilation on the airway system, understanding the molecular mechanisms and the interplay between tidal volume and breathing frequency in the regulation of inflammatory gene expression in airway smooth muscle as well as other cells in the airway system may lead to rational design of ventilation regimens that maximize the beneficial effects while minimizing the inflammatory effects of lung ventilation.

Signaling kinases such as p38 MAPK and IKK (regulator of NF- κ B activation) have been targeted for controlling airway inflammation based on the recognition that these kinases are involved in the regulation of inflammatory gene expression [2, 77, 80, 85]. COX and NF- κ B have also been identified as therapeutic targets in inflammation [51]. However, some transcription factors such as NF- κ B are critical for tissue and organ homeostasis, so that long-term general suppression or overexpression of transcription factors for the treatment of airway inflammation may lead to severe side effects on the homeostasis of other organ systems [142].

Despite the tremendous efforts on targeting single molecules in the treatment of inflammatory airway diseases such as asthma, it is becoming clear in recent years that a single-target approach is unlikely to be effective [14, 36, 170]. Recent analysis of knockout mice studies even casts doubts on the linear model of IgE production, airway eosinophilia, and airway hyperresponsiveness in asthma [106]. These analyses suggest an alternative approach of targeting multiple cell types and/or mediators in the treatment of airway inflammation. This approach requires in-depth understanding of the inflammatory network in the airway system [63]. From the systems point of view, airway inflammation may be considered as a pathophysiological transition from a homeostatic state to a disease state as a result of changes in multiple cells and multiple mediators in the inflammatory network of the airway system. Given the extensive literature reviewed here, it seems timely to consider airway smooth muscle as an integral component of the inflammatory network in the airway system and possibly a therapeutic target of airway inflammation.

ACKNOWLEDGMENTS

I thank Dr. Donald Jackson for his careful reading of a draft of this manuscript. This study was supported by National Heart, Lung, and Blood Institute Grant HL-52714.

ABBREVIATIONS

CCL	=	Chemokine C-C motif ligand
COX	=	Cyclooxygenase
CXCL	=	C-X-C ligand
IFN	=	Interferon
IL	=	Interleukin
RANTES	=	Regulated on activation, normal T cell expressed and secreted
TGF	=	Transforming growth factor
TNF	=	Tumor necrosis factor

REFERENCES

- [1] Abraham, W.M., Scuri, M., Farmer, S.G. *Eur. J. Pharmacol.*, **2006**, 533, 215.
- [2] Adcock, I.M., Chung, K.F., Caramori, G., Ito, K. *Eur. J. Pharmacol.*, **2006**, 533, 118.
- [3] Aderem, A., Smith, K.D. *Semin. Immunol.*, **2004**, 16, 55.
- [4] Agrawal, D.K. *Clin. Exp. Allergy*, **2004**, 34, 1342.
- [5] Agell, N., Bachs, O., Rocamora, N., Villalonga, P. *Cell. Signal.*, **2002**, 14, 649.
- [6] Akdis, C.A., Simons, F.E. *Eur. J. Pharmacol.*, **2006**, 533, 69.
- [7] Allen, M., Svenson, L., Roach, M., Hambor, J., McNeish, J., Gabel, C.A. *J. Exp. Med.*, **2000**, 191, 859-869.
- [8] Ammit, A.J.; Hoffman, R.K.; Amrani, Y.; Lazaar, A.L.; Hay, D.W.P.; Torphy, T.J.; Penn R.B.; Panettieri, R.A., Jr. *Am. J. Respir. Cell Mol. Biol.*, **2000**, 23, 794.
- [9] Ammit, A.J., Lazaar, A.L., Irani, C., O'Neill, G.M., Gordon, N.D., Amrani, Y., Penn, R.B., Panettieri, R.A. Jr. *Am. J. Respir. Cell Mol. Biol.*, **2002**, 26, 465.
- [10] Amrani, Y., Ammit, A.J., Panettieri, R.A. *Mol. Pharmacol.*, **2001**, 60, 646.
- [11] Anderson, M.E. *Pharmacol. Ther.*, **2005**, 106, 39.
- [12] Bachar, O., Rose, A.C., Adner, M., Wang, X., Prendergast, C.E., Kempf, A., Shankley, N.P., Cardell, L.O. *Br. J. Pharmacol.*, **2005**, 144, 220.
- [13] Barlow, C.A., Rose, P., Pulver-Kaste, R.A., Lounsbury, K.M. *J. Physiol.*, **2006**, 570, 59.
- [14] Barnes, P.J. *Nat. Rev. Drug Discov.*, **2004**, 3, 831.
- [15] Belmonte, K.E. *Proc. Am. Thorac. Soc.*, **2005**, 2, 297.
- [16] Blumchen, K., Gerhold, K., Thorade, I., Seib, C. Wahn, U., Hamelmann, E. *Clin. Exp. Allergy*, **2004**, 34, 1124.
- [17] Borish, L.C., Steinke, J.W. *J. Allergy Clin. Immunol.*, **2003**, 111, S460.
- [18] Bregeon, F., Roch, A., Delpierre, S., Ghigo, E., Autillo-Touati, A., Kajikawa, O., Martin, T.R., Pugin, J., Portugal, H., Auffray, J.-P., Jammes, Y. *Respir. Physiol. Neurobiol.*, **2002**, 132, 191.
- [19] Carey, M.A., Germolec, D.R., Langenbach, R., Zeldin, D.C.. *Prostaglandins Leukot. Essent. Fatty Acids*, **2003**, 69, 157.
- [20] Casola, A., Henderson, A., Liu, T., Garofalo, R.P., Brasier, A.R. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **2002**, 283, L1280.
- [21] Caulfield, M.P.; Birdsall, N.J.M. *Pharmacol. Rev.*, **1998**, 50, 279.
- [22] Chung, K.F., Patel, H.J., Fadol, E.J., Rousell, J., Haddad, E.B., Jose, P.J., Mitchell, J., Belvisi, M. *Br. J. Pharmacol.*, **1999**, 127, 1145.
- [23] Conrad, S.A., Zhang, S., Arnold, T.C., Scott, L.K., Carden, D.L. *Crit. Care Med.*, **2005**, 33, 835.
- [24] Copland, I.B., Kavanagh, B.P., Engelberts, D., McKerlie, C., Belik, J., Post, M. *Am. J. Respir. Crit. Care Med.*, **2003**, 168, 1051.
- [25] Corteling, R.L., Li, S., Giddings, J., Westwick, J., Poll, C., Hall, I.P. *Am. J. Respir. Cell. Mol. Biol.*, **2004**, 30, 145.
- [26] Coulson, F.R., Fryer, A.D. *Pharmacol. Ther.*, **2003**, 98, 59.
- [27] Cuschieri, J., Bulger, E., Garcia, I., Jelacic, S., Maier, R.V. *Shock*, **2005**, 23, 99.
- [28] Dalwalwadi, H., Krysan, K., Heue-Voure'h, N., Dohadwala, M., Elashoff, D., Sharma, S., Cacalano, N., Lichtenstein, A., Dubinett, S. *Clin. Cancer Res.*, **2005**, 11, 7674.
- [29] De Koninck, P., Schulman, H. *Science*, **1998**, 279, 227.
- [30] Doganci, A., Sauer, K., Karwot, R., Finotto, S. *Clin. Rev. Allergy Immunol.*, **2005**, 28, 257.
- [31] dos Santos, C.C., Han, B., Andrade, C.F., Bai, X., Uhlig, S., Hubmayr, R., Tsang, M., Lodyga, M., Keshavjee, S., Slutsky, A.S., Liu, M. *Physiol. Genomics*, **2004**, 19, 331.
- [32] Droogmans, L., Cludts, I., Cleuter, Y., Kettmann, R., Burny, A. *DNA Seq.*, **1992**, 3, 115.
- [33] Dupont, G., Goldbeter, A. *BioEssays*, **1998**, 20, 607.
- [34] Elias, J.A., Wu, Y., Zheng, T., Panettieri, R. *Am. J. Physiol.*, **1997**, 273, L648.
- [35] Epsen, E., Bonacchi, A., Pastacaldi, S., Valente, A.J., Wenzel, U.O., Tosti-Guerra, C., Pinzani, M., Laffi, G., Abboud, H.E., Gentilini, P., Marra, F. *Hepatology*, **2001**, 33, 713.
- [36] Fernandes, L.B., Goldie, R.G. *Curr. Opin. Pharmacol.*, **2003**, 3, 251.
- [37] Fields, R.D., Lee, P.R., Cohen, J.E. *Cell Calcium*, **2005**, 37, 433.
- [38] Fong, C.Y., Pang, L., Holland, E., Knox, A.J. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **2000**, 279, L201.

- [39] Fredberg, J.J., Inouye, D., Miller, B., Nathan, M., Jafari, S., Raboudi, S.H., Butler, J.P., Shore, S.A. *Am. J. Respir. Crit. Care Med.*, **1997**, *156*, 1752.
- [40] Gerthoffer, W.T., Singer, C.A. *Respir. Physiol. Neurobiol.*, **2003**, *137*, 237.
- [41] Giustizieri, M.L., Albanesi, C., Fluhr, J., Gisoni, P., Norgauer, J., Girolomoni, G. *J. Allergy Clin. Immunol.*, **2004**, *114*, 1176.
- [42] Gosens, R., Bos, I.S.T., Zaagsma, J., Meurs, H. *Am. J. Respir. Crit. Care Med.*, **2005**, *171*, 1096.
- [43] Gosens, R., Nelemans, S.A., Bromhaar, M.M.G., McKay, S., Zaagsma, J., Meurs, H. *Am. J. Respir. Cell Mol. Biol.*, **2003**, *28*, 257.
- [44] Gosens, R., Zaagsma, J., Bromhaar, M.G., Nelemans, A., Meurs, H. *Eur. J. Pharmacol.*, **2004**, *500*, 193.
- [45] Gosling, M., Poll, C., Li, S. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **2005**, *371*, 277.
- [46] Gounni, A.S. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **2006**, *291*, L312.
- [47] Gounni, A.S., Hamid, Q., Rahman, S.M., Hoeck, J., Yang, J. and Shan, L. *J. Immunol.*, **2004**, *173*, 2771.
- [48] Gounni, A.S., Wellemans, V., Yang, J., Bellesort, F., Kassiri, K., Gangloff, S., Guenounou, M., Halayko, A.J., Hamid, Q., Lamkhioued, B. *J. Immunol.*, **2005**, *175*, 2613.
- [49] Gouwy, M., Struyf, S., Proost, P., Van Damme, J. *Cytokine Growth Factor Rev.*, **2005**, *16*, 561.
- [50] Graffar, O., Hamid, Q., Renzi, P.M., Allakhverdi, Z., Molet, S., Hogg, J.C., Shore, S.A., Luster, A.D., Lamkhioued, B. *Am. J. Respir. Crit. Care Med.*, **1999**, *159*, 1933.
- [51] Haefner, B. *Prog. Med. Chem.*, **2005**, *43*, 137.
- [52] Hakonarson, H., Halapi, E., Whelan, R., Gulcher, J., Stefansson, K., Grunstein, M.M. *Am. J. Respir. Cell Mol. Biol.*, **2001**, *25*, 761.
- [53] Hakonarson, H., Maskeri, N., Carter, C., Chuang, S., Grunstein, M.M. *J. Clin. Invest.*, **1999**, *104*, 657.
- [54] Halayko, A.J., Tran, T., Ji, S.Y., Yamasaki, A., Gosens, R. *Curr. Drug Targets*, **2006**, *7*, 525.
- [55] Halbertsma, F.J.J., Vaneker, M., Scheffer, G.J., van der Hoeven, J.G. *Netherlands J. Med.*, **2005**, *63*, 382.
- [56] Hammerschmidt, S., Kuhn, H., Sack, U., Schlenska, A., Gessner, C., Gillissen, A., Wirtz, H. *Am. J. Respir. Cell Mol. Biol.*, **2005**, *33*, 203-210.
- [57] Hanania, N.A., Moore, R.H. *Curr. Drug Targets Inflamm. Allergy*, **2004**, *3*, 271.
- [58] Hardaker, E.L., Bacon, A.M., Carlson, K., Roshak, A.K., Foley, J.J., Schmidt, D.B., Buckley, P.T., Comegys, M., Panettieri, R.A. Jr., Sarau, H.M., Belmonte, K.E. *FASEB J.*, **2004**, *18*, 191.
- [59] Hedges, J.C., Singer, C.A., Gerthoffer, W.T. *Am. J. Respir. Cell Mol. Biol.*, **2000**, *23*, 86.
- [60] Heitsch, H. *Curr. Med. Chem.*, **2002**, *9*, 913.
- [61] Hirst, S.J. *Respir. Physiol. Neurobiol.*, **2003**, *137*, 309.
- [62] Hirst, S.J., Hallsworth, M.P., Peng, Q., Lee, T.H. *Am. J. Respir. Crit. Care Med.*, **2002**, *165*, 1161.
- [63] Holgate, S.T. *Cytokine*, **2004**, *28*, 152.
- [64] Howarth, P.H., Knox, A.J., Amrani, Y., Tliba, O., Panettieri, R.A., Jr., Johnson, M. *J. Allergy Clin. Immunol.*, **2004**, *114*, S32.
- [65] Howe, C.J., LaHair, M.M., Maxwell, J.A., Lee, J.T., Robinson, P.J., Rodriguez-Mora, O., McCubrey, J.A., Franklin, R.A. *J. Biol. Chem.*, **2002**, *277*, 30469.
- [66] Huang, C.-D., Ammit, A.J., Tliba, O., Kuo, H.-P., Penn, R.B., Panettieri, R.A., Jr., Amrani, Y. *J. Biomed. Sci.*, **2005**, *12*, 763.
- [67] Huang, C.-D., Tliba, O., Panettieri, R.A., Jr., Amrani, Y. *Am. J. Respir. Cell Mol. Biol.*, **2003**, *28*, 330.
- [68] Hudmon, A., Schulman H. *Biochem. J.*, **2002**, *364*, 593.
- [69] Iqbal, J., Zaidi, M. *Biochem. Biophys. Res. Commun.*, **2005**, *328*, 751.
- [70] Iourgenko, V., Zhang, W., Mickanin, C., Daly, I., Jiang, C., Hexham, J.M., Orth, A.P., Miraglia, L., Meltzer, J., Garza, D., Chirn, G.-W., McWhinnie, E., Cohen, D., Skelton, J., Terry, R., Yu, Y., Bodian, D., Buxton, F.P., Zhu, J., Song, C., Labow, M.A. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 12147.
- [71] Jarai, G., Sukkar, M., Garrett, S., Duroudier, N., Westwick, J., Adcock, I., Chung, K.F. *Eur. J. Pharmacol.*, **2004**, *497*, 255.
- [72] Jia, Y., Wang, X., Varty, L., Rizzo, C.A., Yang, R., Correll, C.C., Phelps, P.T., Egan, R.W., Hey, J.A. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **2004**, *287*, L269.
- [73] Joad, J., Casale, T.B. *Ann. Allergy*, **1988**, *61*, 1.
- [74] Johannessen, M., Delghandi, M.P., Moens, U. *Cell. Signal.*, **2004**, *16*, 1211.
- [75] John, M., Au, B.-T., Jose, P.J., Lim, S., Saunders, M., Barnes, P.J., Mitchell, J.A., Belvisi, M.G., Chung, K.F. *Am. J. Respir. Cell Mol. Biol.*, **1998**, *18*, 84.
- [76] Johnson, M. *Proc. Am. Thorac. Soc.*, **2005**, *2*, 320.
- [77] Kaminska, B. *Biochim. Biophys. Acta.*, **2005**, *1754*, 253.
- [78] Kanazawa, H. *Curr. Opin. Pulm. Med.*, **2006**, *12*, 60.
- [79] Kanefsky, J., Lenburg, M., Hai, C.-M. *Am. J. Respir. Cell Mol. Biol.*, **2006**, *34*, 417.
- [80] Karin, M. *Proc. Am. Thorac. Soc.*, **2005**, *2*, 386.
- [81] Kemi, C., Grunewald, J., Eklund, A., Hoglund, C.O. *Respir. Res.*, **2006**, *7*, 18.
- [82] Koyama, S., Rennard, S.I., Robbins, R.A. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **1992**, *262*, L466.
- [83] Kozma, G.T., Losonczy, G., Keszei, M., Komlosi, Z., Buzas, E., Pallinger, E., Appel, J., Szabo, T., Magyar, P., Falus, A., Szalai, C. *Int. Immunol.*, **2003**, *15*, 963.
- [84] Kumar, A., Knox, A.J., Boriek, A.M. *J. Biol. Chem.*, **2003**, *278*, 18868.
- [85] Kumar, S., Boehm, J., Lee, J.C. *Nat. Rev. Drug Discov.*, **2003**, *2*, 717.
- [86] Kupzig, S., Walker, S.A., Cullen, P.J. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*, 7577.
- [87] Lahiri, T., Laporte, J.D., Moore, P.E., Panettieri, R.A., Jr., Shore, S.A. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **2001**, *280*, L1225.
- [88] Laitinen, A., Lindqvist, A., Halme, M., Altraja, A., Laitinen, L.A. *J. Allergy Clin. Immunol.*, **2005**, *115*, 259.
- [89] Laporte, J.D., Moore, P.E., Lahiri, T., Schwartzman, I.N., Panettieri, R.A. Jr., Shore, S.A. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **2000**, *279*, L932.
- [90] Lazaar, A.L., Panettieri, R.A. *J. Allergy Clin. Immunol.*, **2005**, *116*, 488.
- [91] Leeb-Lundberg, L.M.F., Marceau, F., Muller-Esterl, W., Pettibone, D.J., Zuraw, B.L. *Pharmacol. Rev.*, **2005**, *57*, 77.
- [92] Li, Q., Verma, I.M. *Nat. Rev. Immunol.*, **2002**, *2*, 725.
- [93] Lin, C.C., Hsiao, L.D., Chien, C.S., Lee, C.W., Hsieh, J.T., Yang, C.M. *Cell. Signal.*, **2004**, *16*, 597.
- [94] Liu, B., Whisler, R.L. *J. Interferon Cytokine Res.*, **1998**, *18*, 999.
- [95] Liu, S.F., Malik, A.B. *Am. J. Physiol. Cell Mol. Physiol.*, **2006**, *290*, L622.
- [96] Loudon, J.A., Sooranna, S.R., Bennett, P.R., Johnson, M.R. *Mol. Hum. Reprod.*, **2004**, *10*, 895.
- [97] Luo, S.F., Lin, W.N., Yang, C.M., Lee, C.W., Liao, C.H., Leu, Y.L., Hsiao, L.D. *Cell. Signal.*, **2006**, *18*, 1201.
- [98] MacGlashan, D. Jr. *J. Allergy Clin. Immunol.*, **2003**, *112*, S53.
- [99] Macian, F. *Nat. Rev. Immunol.*, **2005**, *5*, 472.
- [100] Marone, G., Granata, F., Spadaro, G., Genovese, A., Triggiani, M. *J. Allergy Clin. Immunol.*, **2003**, *112*, S83.
- [101] Martinez-Matinez, S., Redondo, J.M. *Curr. Med. Chem.*, **2004**, *11*, 997.
- [102] Matsubara, M., Tamura, T., Ohmori, K., Hasegawa, K. *Biochem. Pharmacol.*, **2005**, *69*, 433.
- [103] McKay, S., Bromhaar, M.M.G., de Jongste, J.C., Hoogsteden, H.C., Saxena, P.R., Sharma, H.S. *Mediators Inflamm.*, **2001**, *10*, 135.
- [104] McKay, S., Hirst, S.J., Bertrand-de Haas, M., de Jongste, J.C., Hoogsteden, H.C., Saxena, P.R., Sharma, H.S. *Am. J. Respir. Cell Mol. Biol.*, **2000**, *23*, 103.
- [105] Mishra, J.P., Mishra, S., Gee, K., Kumar, A. *J. Biol. Chem.*, **2005**, *280*, 26825.
- [106] Moffatt, J.D. *Pharmacol. Ther.*, **2005**, *107*, 343.
- [107] Molimard, M., Martin, C.A., Naline, E., Hirsch, A., Advenier, C. *Am. J. Respir. Crit. Care Med.*, **1994**, *149*, 123.
- [108] Moore, P.E., Church, T.L., Chism, D.D., Panettieri, R.A., Jr., Shore, S.A. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **2002**, *282*, L847.
- [109] Nie, M., Knox, A.J., Pang, L. *J. Immunol.*, **2005**, *175*, 478.
- [110] Nie, M., Pang, L., Inoue, H., Knox, A.J. *Mol. Cell. Biol.*, **2003**, *23*, 9233.
- [111] Nockher, W.A., Renz, H. *J. Allergy Clin. Immunol.*, **2006**, *117*, 67.
- [112] Oltmanns, U., Issa, R., Sukkar, M.B., John, M., Chung, K.F. *Br. J. Pharmacol.*, **2003**, *139*, 1228.
- [113] Ong, H.L. and Barritt, G.J. *Respirology*, **2004**, *9*, 448.
- [114] Ong, H.L., Chen, J., Chataway, T., Brereton, H., Zhang, L., Downs, T., Tsiokas, L., Barritt, G. *Biochem. J.*, **2002**, *364*, 641.
- [115] Oudin, S., Pugin, J. *Am. J. Respir. Cell Mol. Biol.*, **2002**, *27*, 107-114.

- [116] Owens, G.K., Kumar, M.S., Wamhoff, B.R. *Physiol. Rev.*, **2004**, *84*, 767.
- [117] Owsianik, G., Talavera, K., Voets, T., Nilius, B. *Ann. Rev. Physiol.*, **2006**, *68*, 685.
- [118] Packard, K.A., Khan, M.M. *Int. Immunopharmacol.*, **2003**, *3*, 909.
- [119] Panettieri, R.A., Jr. *Proc. Am. Thorac. Soc.*, **2004**, *1*, 231.
- [120] Panettieri, R.A., Yadvish, P.A., Kelly, A.M., Rubinstein, N.A., Kotlikoff, M.I. *Am. J. Physiol.*, **1990**, *259*, L365.
- [121] Pang, L., Knox, A.J. *J. Immunol.*, **1998**, *161*, 2509.
- [122] Pang, L., Knox, A.J. *Am. J. Physiol.*, **1997**, *273*, L1132.
- [123] Pang, L., Knox, A.J. *Am. J. Respir. Cell. Mol. Biol.*, **2000**, *23*, 79.
- [124] Pang, L., Knox, A.J. *FASEB J.*, **2001**, *15*, 261.
- [125] Pang, L., Nie, M., Corbett, L., Donnelly, R., Gray, S., Knox, A.J. *FASEB J.*, **2002**, *16*, 1435.
- [126] Park, G.Y., Christman, J.W. *Am. J. Physiol.*, **2006**, *290*, 797.
- [127] Parsons, P.E., Eisner, M.D., Thompson, B.T., Matthay, M.A., Ancukiewicz, M., Bernard, G.R., Wheeler, A.P., NHLBI ARDS Clinical Trials Network. *Crit. Care Med.*, **2005**, *33*, 1.
- [128] Pascual, R.M., Carr, E.M., Seeds, M.C., Guo, M., Panettieri, R.A. Jr., Peters, S.P., Penn, R.B. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **2006**, *290*, L501.
- [129] Pease, J.E., Sabroe, I. *Am. J. Respir. Med.*, **2002**, *1*, 19.
- [130] Pelaia, G., Cuda, G., Vatrella, A., Gallelli, L., Caraglia, M., Marra, M., Abbruzzese, A., Caputi, M., Maselli, R., Costanzo, F.S., Marsico, S.A. *J. Cell. Physiol.*, **2005**, *202*, 642.
- [131] Peng, Q., Matsuda, T., Hirst, S.J. *Am. J. Respir. Crit. Care Med.*, **2004**, *169*, 596.
- [132] Petkova, D.K., Pang, L., Range, S.P., Holland, E., Knox, A.J. *Clin. Exp. Allergy*, **1999**, *29*, 965.
- [133] Pinhu, L., Whitehead, T., Evans, T., Griffiths, M. *Lancet*, **2003**, *361*, 332.
- [134] Plotz, F.B., Slutsky, A.S., van Vught, A.J., Heijnen, C.J. *Intens. Care Med.*, **2004**, *30*, 1865.
- [135] Poff, C.D., Balazy, M. *Curr. Drug Targets Inflamm. Allergy*, **2004**, *3*, 19.
- [136] Prado, G.N., Taylor, L., Zhou, X., Ricupero, D., Mierke, D.F., Polgar, P. *J. Cell. Physiol.*, **2002**, *193*, 275.
- [137] Pugin, J., Dunn, I., Jolliet, P., Tassaux, D., Magnenat, J.-L., Nicod, L.P., Chevrolet, J.-C. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **1998**, *275*, L1040.
- [138] Pulver-Kaste, R.A., Barlow, C.A., Bond, J., Watson, A., Penar, P.L., Tranmer, B., Lounsbury, K.M. *Am. J. Physiol. Heart Circ. Physiol.*, **2006** (in Press)
- [139] Racke, K., Matthiesen, S. *Pulm. Pharmacol. Ther.*, **2004**, *17*, 181.
- [140] Rahman, M.S., Yang, J., Shan, L.Y., Unruh, H., Yang, X., Halayko, A.J., Gounni, A.S. *Clin. Immunol.*, **2005**, *115*, 268.
- [141] Rolin, S., Masereel, B., Dogne, J.-M. *Eur. J. Pharmacol.*, **2006**, *533*, 89.
- [142] Roth, M., Black, J.L. *Curr. Drug Targets*, **2006**, *7*, 589.
- [143] Saklatvala, J. *Curr. Opin. Pharmacol.*, **2004**, *4*, 372.
- [144] Sato, E., Koyama, S., Okubo, Y., Kubo, K., Sekiguchi, M. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **1998**, *274*, L970.
- [145] Schieven, G.L. *Curr. Top. Med. Chem.*, **2005**, *5*, 921.
- [146] Scichilone, N., Togias, A. *Curr. Allergy Asthma Rep.*, **2004**, *4*, 166.
- [147] Singer, C.A., Baker, K.J., McCaffrey, A., AuCoin, D.P., Dechert, M.A., Gerthoffer, W.T. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **2003**, *285*, L1087.
- [148] Singer, C.A., Salinthon, S., Baker, K.J., Gerthoffer, W.T. *BioEssays*, **2004**, *26*, 646.
- [149] Strom, T.B., Sytkowski, A.J., Carpenter, C.B., Merrill, J.P. *Proc. Natl. Acad. Sci. USA*, **1974**, *71*, 1330.
- [150] Sukkar, M.B., Issa, R., Xie, S., Oltmanns, U., Newton, R., Chung, K.F. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **2004**, *287*, L1230.
- [151] Syed, F., Panettieri, R.A., Jr., Thiba, O., Huang, C., Li, K., Bracht, M., Amegadzie, B., Griswold, D., Li, L., Amrani, Y. *Respir. Res.*, **2005**, *6*, 9.
- [152] Thorneloe, K.S. and M.T. Nelson. *Can. J. Physiol. Pharmacol.*, **2005**, *83*, 215.
- [153] Tintinger, G., Steel, H.C., Anderson, R. *Clin. Exp. Immunol.*, **2005**, *141*, 191.
- [154] Togias, A. *J. Allergy Clin. Immunol.*, **2003**, *112*, S60.
- [155] Triggiani, M., Gentile, M., Secondo, A., Granata, F., Oriente, A., Tagliatela, M., Annunziato, L., Marone, G. *J. Immunol.*, **2001**, *166*, 4083.
- [156] Uhlig, S., Uhlig U. *Trends Pharmacol. Sci.*, **2004**, *25*, 592.
- [157] Vanaudenaerde, B.M., Wuyts, W.A., Dupont, L.J., Van Raemdonck, D.E., Demedts, M.M., Verleden, G.M. *J. Heart Lung Transplant.*, **2003**, *22*, 1280.
- [158] Vignola, A.M., Grutta, S.L., Chiappara, G., Benkeder, A., Bellia, V., Bonsignore, G. *Paediatr. Respir. Rev.*, **2002**, *3*, 41.
- [159] von Bethmann, A.N., Brasch, F., Nusing, R., Vogt, K., Volk, H.D., Muller, K.-M., Wendel, A., Uhlig, S. *Am. J. Respir. Crit. Care Med.*, **1998**, *157*, 263.
- [160] von der Ahe, D., Pearson, D., Nagamine, Y. *Nucleic Acids Res.*, **1990**, *18*, 1991.
- [161] Wamhoff, B.R., Bowles, D.K., Owens, G.K. *Circ. Res.*, **2006**, *98*, 868.
- [162] Watson, M.L., Grix, S.P., Jordan, N.J., Place, G.A., Dodd, S., Leithead, J., Poll, C.T., Yoshimura, T., Westwick, J. *Cytokine*, **1998**, *10*, 346.
- [163] Wessler, I.K., Kirkpatrick, C.J. *Pulmonary Pharmacol. Ther.*, **2001**, *14*, 423.
- [164] White, T.A., Xue, A., Chini, E.N., Thompson, M., Sieck, G.C., Wylam, M.E. *Am. J. Respir. Cell. Mol. Biol.*, **2006**, doi:10.1165/rcmb.2006-0003OC.
- [165] Wilson, M.R., Choudhury, S., Goddard, M.E., O'Dea, K.P., Nicholson, A.G., Takata, M. *J. Appl. Physiol.*, **2003**, *95*, 1385.
- [166] Wilson, M.R., Choudhury, S., Takata, M. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **2005**, *288*, L599.
- [167] Wu, G.-Y., Deisseroth, K., Tsien, R.W. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*, 2808.
- [168] Wuyts, W.A., Vanaudenaerde, B.M., Dupont, L.J., Demedts, M.G., Verleden, G.M. *Eur. Respir. J.*, **2003**, *22*, 220.
- [169] Wuyts, W.A., Vanaudenaerde, B.M., Dupont, L.J., Van Raemdonck, D.E., Demedts, M.G., Verleden, G.M. *J. Heart Lung Transplant.*, **2005**, *24*, 875.
- [170] Yamagata, T., Ichinose, M. *Eur. J. Pharmacol.*, **2006**, *533*, 289.
- [171] Yamauchi, T. *Biol. Pharm. Bull.*, **2005**, *28*, 1342.
- [172] Zampetaki, A., Zhang, Z., Hu, Y., Xu, Q. *Am. J. Physiol. Heart Circ. Physiol.*, **2005**, *288*, H2946.
- [173] Zhu, Y.M., Bradbury, D.A., Pang, L., Knox, A.J. *J. Biol. Chem.*, **2003**, *278*, 29366.
- [174] Zuyderduyn, S., Hiermstra, P.S., Rabe, K.F. *J. Allergy Clin. Immunol.*, **2004**, *114*, 791.