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Review Article

AP-1 as a potential therapeutic target in allergic airways inflammation

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CONTENTS

Abstract

Asthma is characterized by a complex inflammatory response of airways eosinophilia, edema, mucus hypersecretion and hyperactivity that is accompanied by structural changes of the airways, termed airways remodeling. Airways remodeling in asthma results in alterations in the airways epithelium, lamina propria and submucosa, leading to thickening of the airways wall. Bronchoalveolar lavage (BAL), biopsy and autopsy data indicate that the severity of the disease is correlated with increased T-helper cell type 2 (Th2) cytokines (IL-4, IL-5 and IL-13). These changes may predispose asthma patients to exacerbations and even death due to airways obstruction. Over the last decade there has been a growing appreciation that chronic airways inflammation is integral to the development of severe asthma and underlies the development of airways hyperactivity. Consequently, increasing emphasis has been placed on the treatment of the underlying inflammatory component of asthma rather than physiological antagonism of the airways smooth muscle response. Most existing asthma treatments treat either the acute bronchoconstriction (β-agonists) or a portion of the acute inflammatory response (leukotriene antagonists), or they have severe dose-limiting side effects (corticosteroids). In this review, we describe the feasibility of inhibiting a novel drug target, the AP-1 transcription factor, as a therapy for asthma. AP-1 elements and AP-1 activation are associated with the transcription of a variety of Th2 cytokines, as well as other inflammatory mediators.

Introduction

Asthma is a chronic disease that has reached epidemic proportions, with about 200 million individuals affected worldwide (1). This disorder can be life-threatening if not properly managed. Current medications used to control asthma fall into one of the following five groups: 1) inhaled bronchodilator medications; 2) antiinflammatory medications; 3) systemic bronchodilators; 5) systemic corticosteroid drugs; and 5) leukotriene modifiers. These medications do not cure the disease, and debilitating symptoms associated with asthma return soon after treatment is stopped, even after long-term therapy (2, 3). To compound the problem, there has been a lack of new therapeutic agents since the introduction of the leukotriene modifiers in the mid-1990s, with the exception of Xolair (omalizumab), a monoclonal anti-IgE antibody (4). Thus, there is a clear need to identify and validate new molecular targets to reduce and/or prevent airways inflammation and remodeling in asthma.

Asthma

Asthma is characterized by a complex inflammatory response comprised of airways eosinophilia, edema, mucus hypersecretion and hyperactivity that is accompanied by structural changes of the airways, termed airways remodeling (5, 6). As with many diseases, the genetics of asthma are multigenic and complex. Inhaled allergen challenge in allergic patients with asthma provokes an immediate airways hypersensitivity reaction, the early-phase airways response (EAR), which is frequently followed several hours later by a delayed airways reaction, the late-phase airways response (LAR). After recovery from the LAR, there is an increase in acquired airways hyperreactivity (AHR) to agents such as methacholine that may persist for several days. Following allergen challenge, the immediate phase of bronchoconstriction in the EAR appears to be in large part dependent upon IgE-mediated mast cell degranulation, with release of leukotriene C_4 (LTC_4), prostaglandin D_2 (PGD_2) and histamine. The LAR comprises a complex sequence of

events that includes mucus hypersecretion, inflammatory cell recruitment, cytokine secretion, enhanced microvascular permeability and acquisition of AHR (7, 8). Separate genetic components may be involved in the early-phase atopy and in the development of AHR, which appears to be acquired through airways remodeling. *Post mortem* studies and investigations using fiber optic bronchoscopy with airways sampling by lavage and biopsy have revealed mucosal inflammatory changes similar to those in the LAR, even in the absence of provoking allergen (8). Hence, over the last decade there has been a growing appreciation that chronic airways inflammation is integral to the development of severe asthma and underlies the development of AHR (7, 8). Consequently, increasing emphasis is being placed on the treatment of the underlying inflammatory component of asthma rather than physiological antagonism of the airways smooth muscle response (9).

Airways inflammation in asthma

Bronchoalveolar lavage (BAL), biopsy and autopsy data indicate that asthmatics have eosinophilia, epithelial cell disruption, subepithelial collagen deposition, smooth muscle and mucus gland hyperplasia, increased numbers of $CD4^+$ T-helper (Th) cells and a corresponding increase in T-helper cell type 2 (Th2) cytokines (IL-4, IL-5 and IL-13), but not Th1 cytokines, such as interferon gamma or IL-2 (10).

Th2 cytokines in asthma

The $CD4^+$ T-cell is essential for the chronic inflammation of asthma. $CD4^+$ T-cells infiltrate the airways of asthmatic subjects, and antigen challenge recruits additional $CD4^+$ T-cells into the airways. $CD4^+$ T-cells in asthmatic patients exhibit a Th2 phenotype, with secretion of IL-4, IL-5 and IL-13. In animal models, depletion of $CD4^+$ T-cells by pretreatment with a specific monoclonal antibody prior to allergen challenge – but after allergen sensitization – prevents AHR and eosinophil recruitment. Allergic airways responses including AHR and eosinophilia can be adoptively transferred with antigen-primed $CD4^+$, but not $CD8^+$ T-cells in Brown Norway rats, and with sensitized T-cells in mice. Potential immunopathological roles for Th2 cells in asthma have been hypothesized based in part on the roles that IL-4, IL-5 and IL-13 play in the stimulation of IgE production, mucosal mastocytosis and eosinophilia (11). Activating protein-1 (AP-1) elements and AP-1 activation are associated with the transcription of a variety of Th1 and Th2 cytokines (12).

Airways remodeling and fibrosis in asthma

Airways remodeling is defined as irreversible structural changes in the asthmatic airways and is considered to be a major determinant of morbidity and mortality in asthma. The pathogenesis of airways remodeling in patients with chronic asthma is unclear. Bronchial biopsies demonstrate thickening of the subepithelial lamina reticularis in the airways in patients with asthma compared to normal controls (13). Morphometric analyses of autopsied lungs from asthmatics demonstrate a marked increase in airways smooth muscle and goblet cells compared to nonasthmatic controls (14). Remodeling involves extracellular matrix (ECM) accumulation and fibrosis in the airways. Particularly characteristic of airways remodeling is the excess deposition of collagen, fibronectin, laminin and tenascin found in the interstitium beneath the basement membrane in patients with asthma (15).

Oxidant/antioxidant imbalance in asthma

Another hallmark of asthma is the oxidant/antioxidant imbalance. Reactive oxygen species (ROS) released by eosinophils and other leukocytes infiltrating the airways play an important role in the airways tissue injury and remodeling process observed in asthma. These ROS include superoxide, hydrogen peroxide (H_2O_2), hydroxyl radicals and nitric oxide (NO) (16). The major eosinophil granule protein by mass is eosinophil peroxidase (EPO). During the respiratory burst, eosinophils and other leukocytes release superoxide and its dismutation product, H_2O_2. The oxidative action of H_2O_2 on target cells is markedly amplified by its EPO-mediated conversion to highly reactive hypophalous acids. In contrast to neutrophil myeloperoxidase (MPO), which uses chloride as a substrate, EPO preferentially utilizes bromide as a substrate to form brominated oxidants. There is an increase in 3-bromotyrosine, an indicator of protein modification by reactive brominating oxidants, at baseline in bronchoalveolar lavage fluid (BALF) of asthmatics compared to nonasthmatics, with a further increase in BALF of asthmatics after segmental allergen challenge (17, 18). Eosinophils also convert NO to a more potent reactive nitrogen species, such as peroxynitrite (ONOO), formed by the interaction of NO and oxygen and 3-nitrotyrosine, which is detected in airways tissue (19, 20) and BALF (17) of asthmatics. Co-localization of EPO with 3-nitrotyrosine is found in lung tissue from patients with fatal asthma (17). These data suggest that eosinophils are a major source of reactive oxygen and nitrogen species released in the airways in asthma.

Asthmatic patients additionally exhibit decreased vitamin C and vitamin E levels in lung lining fluid, even though blood levels are normal or increased (21). The normal epithelial lining fluid in the lungs has approximately 10 times the concentration of the antioxidant glutathione (GSH) than plasma, with reduced levels found in asthmatic lungs (22-24). Increased amounts of oxidized glutathione are found in the airways of asthmatics, indicating increased oxidative stress (25). However, the mechanism by which increased oxidative stress leads to the molecular hallmarks of acute asthma (*i.e.*, Th2

cytokine release) or chronic asthma and the development of fibrosis has not been elucidated. The development of an oxidant/antioxidant imbalance in the lung may lead to activation of redox-sensitive transcription factors, such as NF-κB and AP-1. For the purpose of this review, we will focus only on AP-1.

Activating protein-1 (AP-1)

AP-1 was one of the first mammalian transcription factors to be identified (26) and belongs to the family of basic domain leucine zipper transcription factors. AP-1 is not a single protein, but consists of a dimer of Jun and Fos family members. Jun family members (c-Jun, Jun B and Jun D) form homo- and heterodimers that recognize a TGAGTCA consensus DNA sequence. Fos family members (c-Fos, Fos B, Fra 1 and Fra 2), which are unable to dimerize with each other, augment transcriptional activation by association with Jun family members (23). AP-1 activity is inducible by a variety of stimuli, including cytokines, growth factors and T-cell activators (27).

AP-1 transcription in asthma

The transcriptional activity of AP-1 is redox-sensitive. Hydrogen peroxide and other ROS increase the expression of AP-1 (28-31). The DNA binding of AP-1 increases with a reduction in critical cysteine residues (Cys^{252} in Jun), or mutation from a cysteine to a serine in v-Jun. The DNA binding to AP-1 is decreased when these residues are oxidized (32).

AP-1 is a proinflammatory element that is believed to be an important contributor to the expression of the Th2 cytokines IL-4, IL-5 and IL-13 (33). AP-1 binding sites are found in the promoter regions of many proinflammatory genes besides Th2 cytokines, including adhesion molecules and cell proliferation growth factors (34-36). The gene for Muc5B, responsible for airways mucus production, contains a putative AP-1 consensus site in its promoter (37). The IL-5 proximal promoter element contains an overlapping binding site for the constitutive binding factor Oct-1 and inducible AP-1 (38). Transcriptional induction has been ascribed to the inducible binding element, since a mutant binding element (which lost constitutive Oct-1 binding but maintained inducible AP-1 binding) exerted 3 times greater transcriptional activity than the wild-type element. The IL-4 promoter exists in multiple allelic forms, and a particular allele has high transcriptional activity. A single nucleotide polymorphism located just upstream of the NFAT (nuclear factor of activated T-cells) site appears responsible for the increased promoter strength and markedly enhances the binding affinity of AP-1 complexes (39).

AP-1 as a potential therapeutic target

Glucocorticoids are the most effective therapy for the long-term control of asthma. Their efficacy appears to be largely due to inhibition of abnormal transcriptional regulation of gene expression (40). Inhibition of the proinflammatory transcription factors via transrepression decreases AP-1, as well as NF-κB, STAT (signal transducer and activator of transcription) and NFAT transcriptional activity (41). Glucocorticoids inhibit the transcription of several cytokines and chemokines that are relevant to asthma, including GM-CSF (granulocyte-macrophage colony-stimulating factor), IL-4, IL-5 and eotaxin (42-45). The reduction of GM-CSF and IL-5, key cytokines for eosinophil survival, leads to apoptotic death of eosinophils (46).

A small percentage of asthmatic patients, however, fail to respond to even high doses of glucocorticoids due to resistance (47). Monocytes and lymphocytes from these patients exhibit a marked reduction in the number of activated glucocorticoid receptors in the nucleus after exposure to glucocorticoids compared to normal individuals (48). These same patients also exhibit a reduced inhibitory response on AP-1 activation and elevated JNK activity (49-52). However, glucocorticoid-resistant asthma patients are not resistant to the endocrine and metabolic effects of glucocorticoids, which are major side effects associated with glucorticoid therapy (53). Corticosteroid-resistant (CR) asthma is associated with increased activity of the proinflammatory transcriptional element AP-1 in peripheral blood mononuclear cells (PBMCs). Significantly increased expression of c-fos, phosphorylated c-jun and phosphorylated Jun *N*-terminal kinase (JNK) was found in CR patients compared to corticosteroid-sensitive (CS) patients (52).

Taken together, these data offer compelling evidence for the clinical validation of inhibition of AP-1 transcription as a therapeutic target in asthma.

Redox regulation of AP-1 transcription and asthma

Redox regulation of transcription is important in a variety of inflammatory disease states, including asthma (54, 55). General antioxidants, such as *N*-acetylcysteine (NAC), have been evaluated in animal models and in human subjects (56, 57). However, controversy exists regarding the beneficial effects of antioxidants on asthma (58). Our knowledge concerning the specific factors involved and the scope of this regulatory process remains in its infancy (32, 59).

Thioredoxin (TRX) was originally isolated in *Escherichia coli* as a hydrogen donor for ribonucleotide reductase (60). It is a small multifunctional protein that has a redox-active disulfide/dithiol with a conserved Cys-Gly-Pro-Cys sequence. The human analogue was identified by Yodoi *et al.* (61) as adult T-cell leukemia-derived factor (ADF), which enhanced the production of IL-2R α-chain in HTLV-1-infected lymphocytes. Thioredoxin is known to translocate from the cytosol to the nucleus under a variety of stress-inducing stimuli and is believed to directly regulate the expression of the AP-1 family of genes through the redox factor Ref-1 (62, 63) and the

Table I: Conserved cysteine residues in the AP-1 family of transcription factors.

Fos/Jun family		
	c-fos	RRERNKMAAA**KCR**NRRRELT
	c-ju	KRMRNRIAAS**KCR**RKLERI

NF-κB family (64). Thioredoxin has been implicated in the reduction of an oxidized form of Cys^{62} (likely the *S*-nitroso species) which is required for full transcriptional activation. Based upon the X-ray structure of a p50 NF-κB homodimer complexed with its oligonucleotide binding site (65, 66), Cys^{62} makes contact with the 3'-phosphate of the oligonucleotide backbone.

Ref-1 is a protein which has both endonuclease and redox activity and is involved in the reduction of conserved cysteine residues in fos and jun (Table I). Gronenborn *et al.* have shown by NMR analysis that peptides derived from NF-κB (67) and Ref-1 (64) can bind to the active site of TRX in an extended-strand conformation. More recently, it has become evident that TRX is part of a superfamily of oxidoreductases that are involved in regulating inflammatory and other processes.

AP-1 and TGF-β in airways fibrosis in asthma

Transforming growth factor-β (TGF-β) is a pleiotropic cytokine involved in cell proliferation and differentiation, apoptosis, deposition of ECM, as well as cell adhesion (68). A role for TGF-β as a key mediator in the development of fibrosis in the airways of patients with chronic asthma may be due to its ability to act as a chemoattractant for fibroblasts, stimulate fibroblast procollagen gene expression and protein synthesis, and inhibit collagen breakdown. TGF-β also stabilizes the ECM by inhibiting the expression of ECM proteases and stimulating the expression of ECM protease inhibitors (69-71). Th2 cytokines, in particular IL-13, induce TGF-β (72). TGF-β, together with other factors, such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF), stimulates the release of ECM proteins from myofibroblasts (73, 74). The fibrinolysis system is essential in ECM accumulation and fibrosis. Inhibition of fibrinolysis results in the accumulation of fibrin and ECM.

Plasminogen activator inhibitor-1 (PAI-1) is a key inhibitor of fibrinolysis. Activated mast cells produce abundant PAI-1. These cells may further increase the PAI-1 level in the airways by activating adjacent fibroblasts with TNF-α and TGF-β. Transcriptional regulation by TGF-β is a complex process that involves crosstalk between different DNA-responsive elements and transcription factors to achieve maximal promoter activation and specificity (75). Of particular note, a subset of TGF-β immediate-response target promoters, including TGF-β_1 and most of the TGF-β ECM genes, mediate their response through synergistic SMAD/AP-1 complexes (76-78). The SMADs are a relatively recently identified family of proteins that effect transcription downstream of various members of the TGF-β superfamily (79). Interestingly, the PAI-1 promoter contains several transcription factor binding sites, including AP-1- (80) and SMAD-binding elements (81), that promote PAI-1 induction by TGF-β (82).

Validation of AP-1 as a target for asthma therapeutics

Mouse model of asthma

Important insights into the mechanisms of allergic airways inflammation and AHR in asthma have come from investigations using animal models. Sensitization to a variety of allergens and subsequent airways challenge with the allergen produce typical features of the asthmatic inflammatory response in mice, rats, guinea pigs and nonhuman primates. From these studies, T-cells and eosinophils have clearly emerged as critical cells in mediating the chronic inflammation of asthma. We and others have utilized mouse models which reproduce key morphological and physiological features of human asthma. Henderson *et al.* developed a protocol for the administration of ovalbumin (OVA) as a model allergen to induce allergen-specific pulmonary disease in normal BALB/c and C57BL/6 mice (83-89). This asthma protocol includes immunization of mice with intraperitoneal (i.p.) OVA in alum adjuvant on days 0 and 14 followed by intranasal (i.n.) challenge with OVA in normal saline on days 14, 25, 26 and 27. Control mice receive alum alone for i.p. injection and normal saline alone for i.n. administrations. On day 28 of this protocol, OVA-treated mice display a disease strikingly similar to allergen-induced human asthma, including increased circulating levels of total and OVA-specific IgE, increased release of LTB_4 and LTC_4 in BALF; marked eosinophil influx into BALF and the pulmonary parenchyma; airways goblet cell hyperplasia with mucus occlusion of small airways; increased expression of Th2 cytokines (IL-4, IL-5 and IL-13) and decreased expression of Th1 cytokines (IL-2 and interferon gamma) in bronchial lymph node tissue; and pulmonary hyperreactivity, as assessed by a significantly more rapid decline in airways conductance and dynamic compliance with increasing doses of methacholine compared to control mice.

Small-molecule regulators of redox-regulated AP-1 transcription

To validate AP-1 as a molecular target for asthma, we investigated the development of novel, specific redox regulators of AP-1 transcription. Based on structural information, an extended-strand templated system that can act as a reversible inhibitor (pseudosubstrate) of redox proteins (89-91) was designed (Fig. 1). It was anticipated

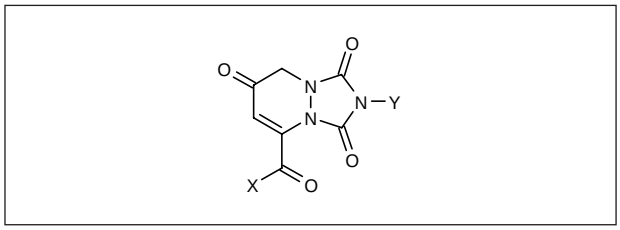

Fig. 1. Pseudosubstrate oxidoreductase template.

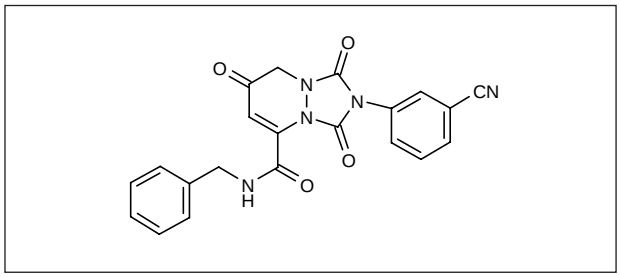

Fig. 2. Molecular structure of the Ref-1/AP-1 inhibitor PNRI-299.

that, through variations in X and Y functionality, specificity for individual redox factors could be achieved. This was based upon the amino acid variations surrounding the conserved cysteine residues of DNA-binding domains within families of transcription factors (92-94).

A limited library of compounds (2 x 6), where X was either $NHCH_3$ or $NHCH_2Ph$ and Y was methyl, phenyl, *m*-NO_2-phenyl, *m*-acetylene, *m*-cyanophenyl or *m*-methylbenzoate, were initially prepared. These analogues were evaluated for their ability to inhibit transcription in transiently transfected human lung epithelial A549 cells by either an NF-κB or an AP-1 reporter. The compound designated PNRI-299 (Fig. 2) selectively inhibited AP-1 transcription with an IC_{50} of 25 μM, without affecting NF-κB transcription at up to 200 μM; this analogue was also nonreactive with TRX at up to 200 μM.

Identification of Ref-1 as the molecular target of PNRI-299

To determine the molecular target(s) of the AP-1 inhibitor PNRI-299, we utilized an affinity chromatography approach. The initial screen indicated that an acylaniline analogue had only slightly decreased activity (~2-fold). To increase the likelihood of affinity-purifying the molecular target(s), we modified the enedione portion by addition of a bromine atom to provide analogue **162-150** (Fig. 3) (95). The affinity reagent **162-150** incorporated an aminocaproic acid (Aca) linker to provide a sufficient distance between the affinity probe and the biotin moiety that was used to bind to the agarose-streptavidin beads. As a negative control, we prepared analogue **162-149**, the design of which was based upon an inactive analogue (Fig. 3). Using affinity chromatography, the molecular target of PNRI-299 was identified as the redox protein Ref-1. Overexpression of Ref-1 could override the effect of PNRI-299 (Fig. 4).

Effect of PNRI-299 on allergic airways inflammation

We next examined the effect of PNRI-299 on airways inflammation in the acute mouse asthma model. Treatment with PNRI-299 significantly decreased the influx of eosinophils into the BALF (Fig. 5a) and airways edema (Fig. 5b) in OVA-treated mice. The OVA-sensitized/challenged mice developed a striking infiltration of the airways by eosinophils and other inflammatory cells and mucus hypersecretion (Fig. 6a) that was not observed in saline-treated controls (Fig. 6c). Treatment with PNRI-299 significantly decreased the influx of eosinophils, monocytes and macrophages (no significant reduction of lymphocytes was observed) into the lung interstitium (Fig. 6b), BALF and airways mucus (Fig. 6b), as well as the edema observed in OVA-treated mice. As assessed by RT-PCR, the gene expression of IL-4 and IL-5 was markedly increased, that of eotaxin was minimally increased and that of CCR3 was unchanged in whole lung tissue of the OVA-sensitized/challenged mice compared to saline-treated controls (96). The increased IL-4 gene expression in the OVA-treated mice was attenuated in a dose-dependent manner by PNRI-299 (0.75-2 mg/kg). However, the increased IL-5 and eotaxin gene expression in the OVA-treated mice was unaffected by either dose of PNRI-299.

Another potential benefit achievable through AP-1 inhibition is a decrease in the cysteinyl leukotriene LTC_4. Cysteinyl leukotrienes modulate contraction in airways

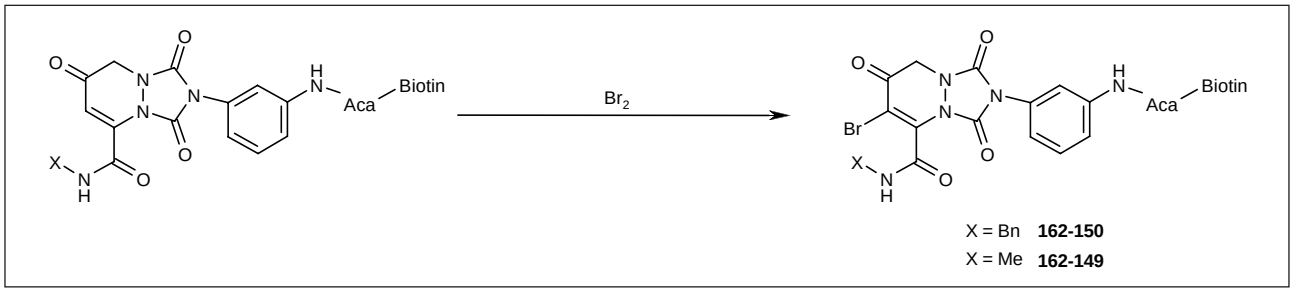

Fig. 3. Affinity probe synthesis: Generation of the affinity reagents **162-150** and **162-149**.

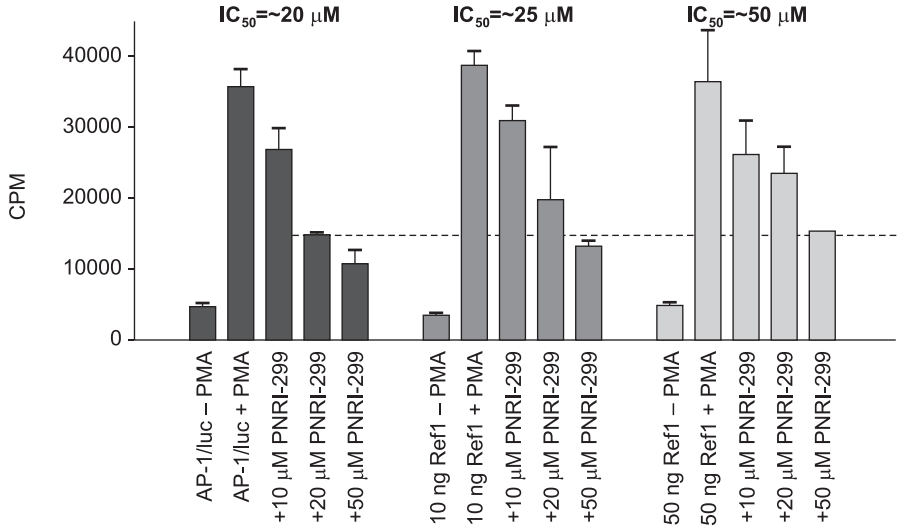

Fig. 4. Identification of Ref-1 as the molecular target of the AP-1 inhibitor PNRI-299. Cotransfection of Ref-1 expression vector in A549 cells transfected with an AP-1-luciferase reporter gene construct.

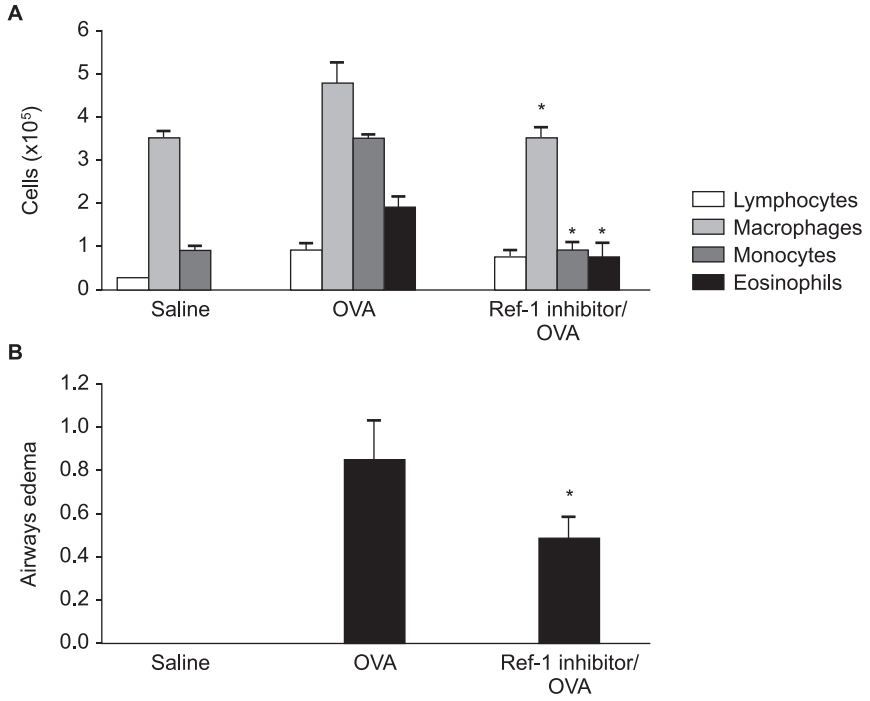

Fig. 5. Reduction of asthmatic response. Bronchoalveolar lavage fluid eosinophils, monocytes, macrophages and lymphocytes (**A**) and airways edema (**B**) were determined in controls (saline) and ovalbumin (OVA)-treated mice in the absence (OVA) or presence (Ref-1 inhibitor/OVA) of PNRI-299. *$p < 0.05$ *vs.* OVA by Student's two-tailed *t*-test.

smooth muscle, promote smooth muscle proliferation, inflammatory cell influx, increase vascular permeability and induce mucus secretion (96-99). LTC_4 synthase is a specific glutathione-*S*-transferase located in the nuclear envelope that converts LTA_4 to LTC_4 in the final step of LTC_4 biosynthesis (100). A recent report described a putative AP-1 element in the LTC_4 synthase proximal promoter (101). Reporter gene assays in THP-1 cells demonstrated a modest reduction in LTC_4 synthase expression induced by PNRI-299. Furthermore, PNRI-299 demonstrated a dose-dependent reduction in LTC_4 synthase expression in the lungs of OVA-sensitized mice (102).

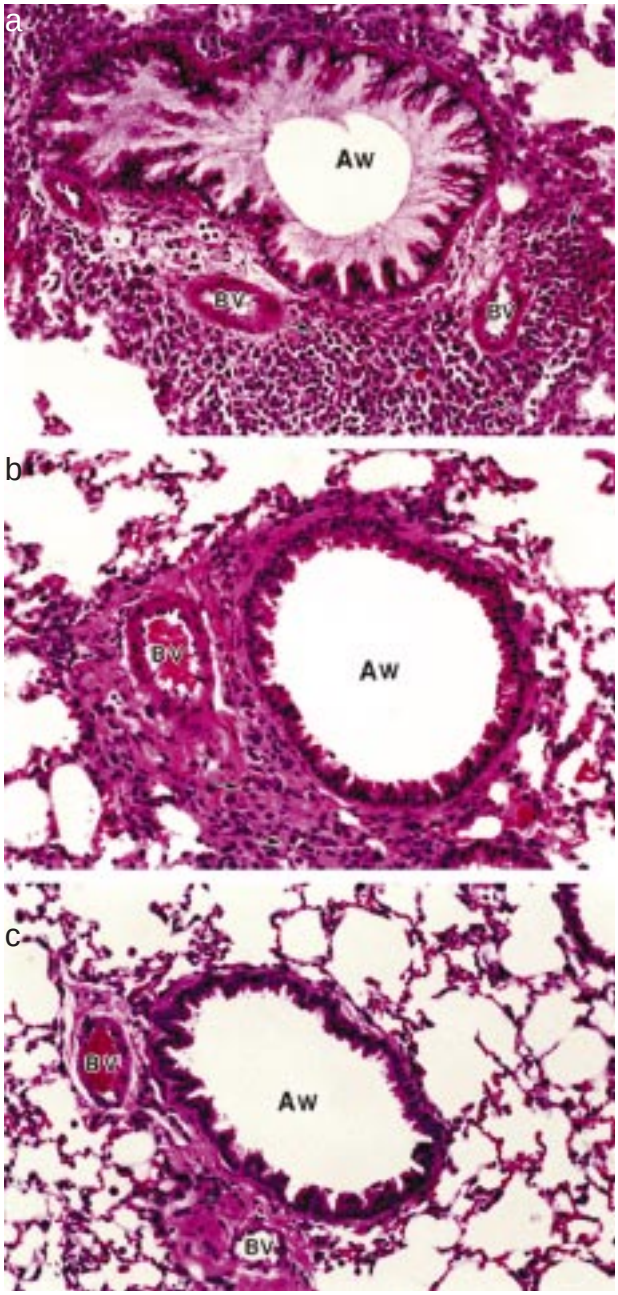

Fig. 6. Ref-1 inhibitor PNRI-299 reduces allergic airways inflammation. (a) Ovalbumin (OVA)-treated mice have a dense inflammatory cell infiltrate (arrows) surrounding the airways (AW) and blood vessels (BV) and mucus hypersecretion not seen in saline controls (c). (b) PNRI-299 (0.75 mg/kg) reduced the airways inflammatory cell infiltration (arrows) and mucus release in OVA-treated mice.

Conclusions

AP-1 was one of the first mammalian transcription factors identified (26). Its activity is induced by a wide array of stimuli, and in turn, it regulates a wide range of cellular processes, including cell proliferation, differentiation, survival and death (103). AP-1 elements are found in the promoter regions of a plethora of inflammatory mediators, cytokines and chemokines. AP-1 transcription is increased during oxidative stress, which accompanies chronic asthma and pulmonary fibrosis (104, 105). Most existing antiasthmatic agents treat the acute bronchoconstriction (β-agonists) or a portion of the acute inflammatory response (leukotriene antagonists), or they have severe dose-limiting side effects (corticosteroids). Increased activation and expression of AP-1 have been demonstrated in the airways of asthmatic patients (106). Inhibition of AP-1 transcription offers the benefit of treating the underlying inflammatory process and reducing the production of inflammatory cytokines and chemokines that are important in chronic asthma, which can eventually lead to pulmonary fibrosis.

Our work (96) demonstrated the efficacy of a novel inhibitor of redox-regulated AP-1 transcription in a murine model of allergic asthma. Furthermore, recently, a small-molecule inhibitor of JNK (SP-600125) demonstrated the ability to reduce eosinophil and lymphocyte infiltration in a rat model of chronic asthma (107). These studies set the stage for further efforts to develop novel, selective, small-molecule inhibitors of AP-1 transcription and for the clinical evaluation of these inhibitors in chronic asthma and pulmonary fibrotic diseases. One caveat is worth noting – as AP-1 transcription is involved in such a broad range of cellular processes, concerns arise as to potential liabilities associated with the systemic use of AP-1 transcriptional regulators. Although clearly a concern, we have demonstrated (96) that specific inhibitors of AP-1 transcription have a selective rather than a global effect on the expression of inflammatory genes. Furthermore, glucocorticoids, which more broadly inhibit gene transcription through both AP-1 and NF-κB, and also activate glucocorticoid-responsive genes and therefore possess potent systemic toxicities, remain a mainstay of antiasthmatic therapy. The questions and concerns raised regarding the prospects for this novel approach to the treatment of asthma and pulmonary fibrosis will hopefully be answered by the further development of selective AP-1 transcriptional regulators and their entry into the clinic.

Acknowledgements

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References

1. Holgate, S.T. *The epidemic of allergy and asthma*. Nature1999, 402: B2-4.

2. Kemp, J. *Recent advances in the management of asthma using leukotriene modifiers*. Am J Resp Med 2003, 2: 139-56.

3. Romagnani, S. *New therapeutic strategies in allergic diseases*. Drugs Today 2003, 39: 849-65.

4. Ames, S.A., Gleeson, C.D., Kirkpatrick, P. *Omalizumab.* Nat Rev Drug Discov 2004, 3(3): 199-200.

5. Barnes, P.J., Chung, K.F., Page, C.P. *Inflammatory mediators of asthma: An update*. Pharmacol Rev 1998, 50: 515-96.

6. Jeffery, P.K. *Structural and inflammatory changes in COPD: A comparison with asthma*. Thorax 1998, 53: 129-36.

7. Chauhan, A.J., Krishna, M.T., Holgate, S.T. *Aetiology of asthma: How public health and molecular medicine work together*. Mol Med Today 1996, 2: 192-7.

8. Howarth, P.H. *The airway inflammatory response in allergic asthma and its relationship to clinical disease.* Allergy 1995, 50(22, Suppl.): 13-21.

9. International Consensus Report on Diagnosis and Treatment of Asthma [see comments]. Clin Exp Allergy 1992, 22(Suppl. 1): 1-172.

10. Kon, O.M., Kay, A.B. *T cells and chronic asthma*. Int Arch Allergy Immunol 1999, 118: 133-5.

11. Street, N.E., Mossman, T.R. *Functional diversity of T lymphocytes due to secretion of different cytokine patterns*. FASEB J 1991, 5: 171-7.

12. Macian, F., Lopez-Rodriguez, C., Rao, A. *Partners in transcription: NFAT and AP-1.* Oncogene 2001, 20(19): 2476-89.

13. Hoshino, M., Nakamura, Y., Sim, J.J. *Expression of growth factors and remodelling of the airway wall in bronchial asthma.* Thorax 1998, 53(1): 21-7.

14. Aikawa, T., Shimura, S., Sasaki, H., Ebina, M., Takishima, T. *Marked goblet cell hyperplasia with mucus accumulation in the airways of patients who died of severe acute asthma attack.* Chest 1992, 101: 916-21.

15. Roche, W.R., Beasley, R., Williams, J.H., Holgate, S.T. *Subepithelial fibrosis in the bronchi of asthmatics.* Lancet 1989, 1: 520-4.

16. Nordberg, J., Arner, E.S.J. *Reactive oxygen species, antioxidants, and the mammalian thioredoxin system.* Free Radic Biol Med 2001, 31: 1287-312.

17. MacPherson, J.C., Comhair, S.A., Erzurum, S.C. et al. *Eosinophils are a major source of nitric oxide-derived oxidants in severe asthma: Characterization of pathways available to eosinophils for generating reactive nitrogen species.* J Immunol 2001, 166: 5763-72.

18. Wu, W., Samoszuk, M.K., Comhair, S.A. et al. *Eosinophils generate brominating oxidants in allergen-induced asthma.* J Clin Invest 2000, 105: 1455-63.

19. Saleh, D., Ernst, P., Lim, S., Barnes, P.J., Giaid, A. *Increased formation of the potent oxidant peroxynitrite in the airways of asthmatic patients is associated with induction of nitric oxide synthase: Effect of inhaled glucocorticoid*. FASEB J 1998, 12(11): 929-37.

20. Wenzel, S.E., Trudeau, J.B., Kaminsky, D.A., Cohn, J., Martin, R.J., Westcott, J.Y. *Effect of 5-lipoxygenase inhibition on bronchoconstriction and airway inflammation in nocturnal asthma.* Am J Resp Crit Care Med 1995, 152(3): 897-905.

21. Kelly, F.J., Mudway, I., Blomberg, A., Frew, A., Sandstrom, T. *Altered lung antioxidant status in patients with mild asthma*. Lancet 1999, 354: 482-3.

22. Dauletbaev, N., Rickmann, J., Viel, K., Buhl, R., Wagner, T.O., Bargon, J. *Glutathione in induced sputum of healthy individuals and patients with asthma.* Thorax. 2001, 56: 13-8.

23. Smith, L.J., Houston, M., Anderson, J. *Increased levels of glutathione in bronchoalveolar lavage fluid from patients with asthma*. Am Rev Resp Dis 1993, 147: 1461-4.

24. Cantin, A.M., North S.L., Hubbard, R.C., Crystal, R.G. *Normal alveolar epithelial lining fluid contains high levels of glutathione*. J Appl Physiol 1987, 63: 152-7.

25. Nadeem, A., Chhabra, S.K., Masood, A., Raj, H.G. *Increased oxidative stress and altered levels of antioxidants in asthma.* J Allergy Clin Immunol 2003, 111: 72-8.

26. Angel, P., Karin, M. *The role of Jun, Fos and the AP-1 complex in cell proliferation and transformation.* Biochem Biophys Acta 1991, 1072: 129-57.

27. Lee, W., Mitchell, P., Tjian, R. *Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements.* Cell 1987, 49: 74-82.

28. Timblin, C.R., Janssen, Y.M., Goldberg, J.L., Mossman, B.T. *GRP78, HSP72/73 and c-Jun stress protein levels in lung epithelial cells exposed to asbestos, cadmium or H_2O_2*. Free Radic Biol Med 1998, 24: 632-42.

29. Singh, N., Aggarwal, S. *The effect of active oxygen generated by xanthine/xanthine oxidase on genes and signal transduction in mouse epidermal JB6 cells*. Int J Cancer 1995, 62: 107-14.

30. Ozolins, T.R., Hales, B.F. *Oxidative stress regulates the expression and activity of transcription factor activator protein-1 in rat conceptus*. J Pharmacol Exp Ther 1997, 280: 1085-93.

31. Nose, K., Shibanuma, M., Kikuchi, K., Kageyama, H., Sakiyama, S., Kuroki, T. *Transcriptional activation of early response genes by hydrogen peroxide in a mouse osteoblastic cell line*. Eur J Biochem 1991, 201: 99-106.

32. Schenk, H., Klein, M., Erdbrugger, W., Droge, W., Schulze-Osthoff, K. *Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-κB and AP-1*. Proc Natl Acad Sci USA 1994, 91: 1672-6.

33. Lavender, P., Cousins, D., Lee, T. *Regulation of Th2 cytokine gene transcription*. Chem Immunol 2000, 78: 16-29.

34. Dalton, T.P., Shertzer H.G., Puga, A. *Regulation of gene expression by reactive oxygen*. Annu Rev Pharmacol Toxicol 1999, 39: 67-101.

35. Nakamura, H., Nakamura, K., Yodoi, J. *Redox regulation of cellular activation.* Annu Rev Immunol 1997, 15: 351-69.

36. Bazan, N.G., Morrelli de Liberti, S.G., Rodriguez de Turco, E.B., Pediconi, M.F. *Free arachidonic and docosahexaenoic acid accumulation in the central nervous system during stimulation*. In: Neural Membranes. G.Y. Sun, N. Banzan, J.Y. Wu, G. Porcellati, A.Y. Sun (Ed.). Humana Press: Clifton, 1983, 123-40.

37. Van, S., Perrais, I.M., Pigny, P., Porchet, N., Aubert, J.P. *Sequence of the 5'-flanking region and promoter activity of the human mucin gene MUC5B in different phenotypes of colon cancer cells.* Biochem J 2000, 348(Pt. 3): 675-86.

38. Schwenger, G.T., Kok, C.C., Arthaningtyas, E., Thomas, M.A., Sanderson, C.J., Mordvinov, V.A. *Specific activation of*

human interleukin-5 depends on de novo synthesis of an AP-1 complex. J Biol Chem 2002, 277: 47022-7.

39. Song, Z., Casolaro, V., Chen, R., Georas, S.N., Monos, D., Ono, S.J. *Polymorphic nucleotides within the human IL-4 promoter that mediate overexpression of the gene*. J Immunol 1996, 156: 424-9.

40. Barnes, P.J. *Anti-inflammatory actions of glucocorticoids: molecular mechanisms*. Clin Sci (Lond) 1998, 94: 557-72.

41. Adcock, I.M. *Glucocorticoid-regulated transcription factors*. Pulm Pharmacol Ther 2001, 14: 211-9.

42. Guyre, P.M., Girard, M.T., Morganelli, P.M., Manganiello, P.D. *Glucocorticoid effects on the production and actions of immune cytokines*. J Steroid Biochem 1988, 30: 89-93.

43. Wu, C.Y., Fargeas, C., Nakajima, T., Delespesse, G. *Glucocorticoids suppress the production of interleukin 4 by human lymphocytes.* Eur J Immunol 1991, 21: 2645-7.

44. Rolfe, F.G., Hughes, J.M., Armour, C.L. Sewell, W.A. *Inhibition of interleukin-5 gene expression by dexamethasone*. Immunology. 1992, 77(4): 494-9.

45. Lilly, C.M., Nakamura, H., Kesselman, H., Nagler-Anderson, C., Asano, K., Garcia-Zepeda, E.A., Rothenberg, M.E., Drazen, J.M., Luster, A.D. *Expression of eotaxin by human lung epithelial cells: Induction by cytokines and inhibition by glucocorticoids*. J Clin Invest 1997, 99(7): 1767-73.

46. Kinloch, R.A., Treherne, J.M., Furness, L.M., Hajimohamadreza, I. *The pharmacology of apoptosis.* Trends Pharmacol Sci 1999, 20: 35-42.

47. Barnes, P.J., Adcock, I.M. *Steroid resistance in asthma*. Quart J Med 1995, 88(7): 455-68.

48. Adcock, I.M., Lane, S.J., Brown, C.R., Peters, M.J., Lee, T.H., Barnes, P.J. *Differences in binding of glucocorticoid receptor to DNA in steroid-resistant asthma.* J Immunol 1995, 154(7): 3500-5.

49. Gonzalez, M.V., Jimenez, B., Berciano, M.T. et al. *Glucocorticoids antagonize AP-1 by inhibiting the activation/phosphorylation of JNK without affecting its subcellular distribution*. J Cell Biol 2000, 150(5): 1199-208.

50. Adcock, I.M., Lane, S.J., Brown, C.R., Lee, T.H., Barnes, P.J. *Abnormal glucocorticoid receptor-activator protein 1 interaction in steroid-resistant asthma*. J Exp Med 1995, 182(6): 1951-8.

51. Lane, S.J., Adcock, I.M., Richards, D., Hawrylowicz, C., Barnes, P.J., Lee, T.H. *Corticosteroid-resistant bronchial asthma is associated with increased c-fos expression in monocytes and T lymphocytes*. J Clin Invest 1998, 102(12): 2156-64.

52. Sousa, A.R., Lane, S.J., Soh, C., Lee, T.H. *In vivo resistance to corticosteroids in bronchial asthma is associated with enhanced phosyphorylation of JUN N-terminal kinase and failure of prednisolone to inhibit JUN N-terminal kinase phosphorylation*. J Allergy Clin Immunol 1999, 104(3, Pt. 1): 565-74.

53. Lane, S.J., Atkinson, B.A., Swaminathan, R., Lee, T.H. *Hypothalamic-pituitary-adrenal axis in corticosteroid-resistant bronchial asthma.* Am J Resp Crit Care Med 1996, 153(2): 557-60.

54. Matthews, J.R., Wakasugi, N., Virelizier, J.L., Yodoi, J., Hay, R.T. *Thioredoxin regulates the DNA binding activity of NF-κB by reduction of a disulphide bond involving cysteine 62.* Nucleic Acids Res 1992, 20: 3821-30.

55. Nakamura, H., Nakamura, K., Yodoi, J. *Redox regulation of cellular activation*. Annu Rev Immunol 1997, 15: 351-69.

56. Millman, M., Millman, F.M., Goldstein, I.M., Mercandetti, A.J. *Use of acetylcysteine in bronchial asthma—Another look.* Ann Allergy 1985, 54: 294-6.

57. Dorsch,W., Auch, E., Powerlowicz, P. *Adverse effects of acetylcysteine on human and guinea pig bronchial asthma in vivo and on human fibroblasts and leukocytes in vitro*. Int Arch Allergy Appl Immunol 1987, 82: 33-9.

58. Neuman, I., Nahum, H., Ben-Amotz, A. *Reduction of exercise-induced asthma oxidative stress by lycopene, a natural antioxidant*. Allergy 2000, 55(12): 1184-9.

59. Jin, D.Y., Chae, H.Z., Rhee, S.G., Jeang, K.T. *Regulatory role for a novel human thioredoxin peroxidase in NF-κB activation.* J Biol Chem 1997, 272: 30952-61.

60. Laurent, T.C., Moore, E.C., Reichard, P. *Enzymatic synthesis of deoxyrinonucleotides. IV. Isolation and characterization of thioredoxin, the hydrogen bond donor from Escherichia coli B.* J Biol Chem 1964, 239: 3436-44.

61. Okamoto, T., Ogiwara, H., Hayashi, T., Mitsui, A., Kawabe, T., Yodoi, J. *Human thioredoxin/adult T cell leukemia-derived factor activates the enhancer binding protein of human immunodeficiency virus type 1 by thiol redox control mechanism*. Int Immunol 1992, 4(7): 811-9.

62. Xanthoudakis, S., Curran, T. *Identification and characterization of Ref-1, a nuclear protein that facilitates AP-1 DNA-binding activity*. EMBO J 1992, 11: 653-65.

63. Hirota, K., Matsui, M., Iwata, S., Nishiyama, A., Mori, K., Yodoi, J. *AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1*. Proc Natl Acad Sci USA 1997, 94(8): 3633-8.

64. Qin, J., Clore, G.M., Kennedy, W.P., Kuszewski, J., Gronenborn, A.M. *The solution structure of human thioredoxin complexed with its target from Ref-1 reveals peptide chain reversal.* Structure 1996, 4(5): 613-20.

65. Muller, C.W., Rey, F.A., Sodeoka, M., Verdine, G.L., Harrison, S.C. *Structure of the NF-κ B p50 homodimer bound to DNA.* Nature 1995, 373(6512): 311-7.

66. Ghosh, G., van Duyne, G., Ghosh, S., Sigler, P.B. *Structure of NF-kappa B p50 homodimer bound to a κB site.* Nature 1995, 373(6512): 303-10.

67. Qin, J., Clore, G.M., Kennedy, W.M., Huth, J.R., Gronenborn, A.M. *Solution structure of human thioredoxin in a mixed disulfide intermediate complex with its target peptide from the transcription factor NFκB.* Structure 1995, 3(3): 289-97.

68. Wahl, S.M., Orenstein, S.M., Chen, W. *TGF-β influences the life and death decisions of T lymphocytes.* Cytokine Growth Factor Rev 2000, 11: 71-9.

69. Duvernelle, C., Freund, V., Frossard, N. *Transforming growth factor-β and its role in asthma.* Pulm Pharmacol Ther 2003, 16(4): 181-96.

70. Kenyon, N.J., Ward, R.W., McGrew, G., Last, J.A. *TGF-β1 causes airway fibrosis and increased collagen I and III mRNA in mice.* Thorax 2003, 58(9): 772-7.

71. Minshall, E.M., Leung, D.Y., Martin, R.J., Song, Y.L., Cameron, L., Ernst, P., Hamid, Q. *Eosinophil-associated*

TGF-β1 mRNA expression and airways fibrosis in bronchial asthma. Am J Resp Cell Mol Biol 1997, 17(3): 326-33.

72. Wen, F.Q., Kohyama, T., Liu, X., Zhu, Y.K., Wang, H., Kim, H.J., Kobayashi, T., Abe, S., Spurzem, J.R., Rennard, S.I. *Interleukin-4- and interleukin-13-enhanced transforming growth factor-β2 production in cultured human bronchial epithelial cells is attenuated by interferon-γ.* Am J Resp Cell Mol Biol 2002, 26(4): 484-90.

73. Kumar, R.K., Herbert, C., Foster, P.S. *Expression of growth factors by airway epithelial cells in a model of chronic asthma: Regulation and relationship to subepithelial fibrosis*. Clin Exp Allergy 2004, 34(4): 567-75.

74. Saito, A., Okazaki, H., Sugawara, I., Yamamoto, K., Takizawa, H. *Potential action of IL-4 and IL-13 as fibrogenic factors on lung fibroblasts in vitro.* Int Arch Allergy Immunol 2003, 132(2): 168-76.

75. Massague, J. *How cells read TGF-β signals.* Mol Cell 2000, 1: 169-77.

76. Wong, C., Rougier-Chapman, E.M., Frederick, J.P., Datto, M.B., Liberati, N.T., Li, J.M., Wang, X.F. *Smad3-Smad4 and AP-1 complexes synergize in transcriptional activation of the c-Jun promoter by transforming growth factor beta.* Mol Cell Biol 1999, 19(3): 1821-30.

77. Chung, K.Y., Agarwal, A., Uitto, J., Mauviel, A. *An AP-1 binding sequence is essential for regulation of the human α2(I) collagen (COL1A2) promoter activity by transforming growth factor-β.* J Biol Chem 1996, 271(6): 3272-8.

78. Kim, S.J., Angel, P., Lafyatis, R., Hattori, K., Kim, K.Y., Sporn, M.B., Karin, M., Roberts. A.B. *Autoinduction of transforming growth factor β1 is mediated by the AP-1 complex*. Mol Cell Biol 1990, 10(4): 1492-7.

79. Hata, A., Shi, Y., Massague, J. *TGF-β signaling and cancer: Structural and functional consequences of mutations in Smads*. Mol Med Today 1998, 4(6): 257-62.

80. Olman, M.A., Hagood, J.S., Simmons, W.L., Fuller, G.M., Vinson, C., White, K.E. *Fibrin fragment induction of plasminogen activator inhibitor transcription is mediated by activator protein-1 through a highly conserved element*. Blood 1999, 94(6): 2029-38.

81. Yingling, J.M., Datto, M.B., Wong, C., Frederick, J.P., Liberati, N.T., Wang, X.F. *Tumor suppressor Smad4 is a transforming growth factor β-inducible DNA binding protein*. Mol Cell Biol 1997, 17(12): 7019-28.

82. Keeton, M.R., Curriden, S.A., van Zonneveld, A.J., Loskutoff, D.J. *Identification of regulatory sequences in the type 1 plasminogen activator inhibitor gene responsive to transforming growth factor β*. J Biol Chem 1991, 266: 23048-52.

83. Henderson, W.R. Jr., Lewis, D.B., Albert, R.K. et al. *The importance of leukotrienes in airway inflammation in a mouse model of asthma*. J Exp Med 1996, 184(4): 1483-94.

84. Henderson, W.R. Jr., Lu, J., Poole, K.M., Dietsch, G.N., Chi, E.Y. *Recombinant human platelet-activating factor-acetylhydrolase inhibits airway inflammation and hyperreactivity in mouse asthma model*. J Immunol 2000, 164(6): 3360-7.

85. Henderson, W.R. Jr., Chi, E.Y., Maliszewski, C.R. *Soluble L-4 receptor inhibits airway inflammation following allergen challenge in a mouse model of asthma*. J Immunol 2000, 164(2): 1086-95.

86. Henderson, W.R. Jr., Chi, E.Y., Albert, R.K., Chu, S.J., Lamm, W.J., Rochon, Y., Jonas, M., Christie, P.E., Harlan, J.M. *Blockade of CD49d (α4 integrin) on intrapulmonary but not circulating leukocytes inhibits airway inflammation and hyperresponsiveness in a mouse model of asthma*. J Clin Invest 1997, 100(12): 3083-92.

87. Oh, S.W., Pae, C.I., Lee, D.K., Jones, F., Chiang, G.K., Kim, H.O., Moon, S.H., Cao, B., Ogbu, C., Jeong, K.W., Kozu, G., Nakanishi, H., Kahn, M., Chi, E.Y., Henderson, W.R. Jr. *Tryptase inhibition blocks airway inflammation in a mouse asthma model*. J Immunol 2002, 168(4): 1992-2000.

88. Zhang, Y., Lamm, W.J., Albert, R.K., Chi, E.Y., Henderson, W.R. Jr., Lewis. D.B. *Influence of the route of allergen administration and genetic background on the murine allergic pulmonary response*. Am J Resp Crit Care Med 1997, 155(2): 661-9.

89. Ogbu, C.O., Qabar, M.N., Boatman, P.D., Urban, J., Meara, J.P., Ferguson, M.D., Tulinsky, J., Lum, C., Babu, S., Blaskovich, M.A., Nakanishi, H., Ruan, F., Cao, B., Minarik, R., Little, T., Nelson, S., Nguyen, M., Gall, A., Kahn, M. *Highly efficient and versatile synthesis of libraries of constrained β-strand mimetics.* Bioorg Med Chem Lett 1998, 8(17): 2321-6.

90. Boatman, P.D., Ogbu, C.O., Eguchi, M., Kim, H.O., Nakanishi, H., Cao, B., Shea, J.P., Kahn, M. *Secondary structure peptide mimetics: Design, synthesis, and evaluation of β-strand mimetic thrombin inhibitors*. J Med Chem 1999, 42(8): 1367-75.

91. Kahn, M., Eguchi, M. *Synthesis of peptides incorporating β-turn inducers and mimetics*. In: Houben-Weyl Methods of Organic Chemistry, Vol. E22: Synthesis of Peptides and Peptidomimetics. M. Goodman, A. Felix, L. Moroder and C. Toniolo (Eds.) Georg Thieine Verlag, Stuttgart/New York, 2003, Ch.12.1.

92. Akamatsu, Y., Ohno, T., Hirota, K., Kagoshima, H., Yodoi, J., Shigesada, K. *Redox regulation of the DNA binding activity in transcription factor PEBP2. The roles of two conserved cysteine residues*. J Biol Chem 1997, 272(23): 14497-500.

93. Kuge, S., Jones, N., Nomoto, A. *Regulation of yAP-1 nuclear localization in response to oxidative stress.* EMBO J 1997, 16(7): 1710-20.

94. Sun, Y., Oberley, L.W. *Redox regulation of transcriptional activators*. Free Radic Biol Med 1996, 21(3): 335-48.

95. Rosania, G.R., Chang, Y.T., Perez, O., Sutherlin, D., Dong, H., Lockhart, D.J., Schultz, P.G. *Myoseverin, a microtubule-binding molecule with novel cellular effects*. Nat Biotechnol 2000, 18(3): 304-8.

96. Nguyen, C., Teo, J.L., Matsuda, A., Eguchi, M., Chi, E.Y., Henderson, W.R. Jr., Kahn, M. *Chemogenomic identification of Ref-1/AP-1 as a therapeutic target for asthma*. Proc Natl Acad Sci USA 2003, 100(3): 1169-73.

97. Bigby, T.D. *Regulation of expression of the 5-lipoxygenase pathway*. Clin Rev Allergy Immunol 1999, 17(1-2): 43-58.

98. Lee, E., Robertson, T., Smith, J., Kilfeather, S. *Leukotriene receptor antagonists and synthesis inhibitors reverse survival in eosinophils of asthmatic individuals*. Am J Resp Crit Care Med 2000, 161(6): 1881-6.

99. Barnes, N.C., Smith, L.J. *Biochemistry and physiology of the leukotrienes.* Clin Rev Allergy Immunol 1999, 17(1-2): 27-42.

100. Bandeira-Melo, C., Weller, P.F. *Eosinophils and cysteinyl leukotrienes*. Prostaglandins Leukot Essent Fatty Acids 2003, 69(2-3): 135-43.

101. Bigby, T.D., Hodulik, C.R., Arden, K.C., Fu, L. *Molecular cloning of the human leukotriene C4 synthase gene and assignment to chromosome 5q35*. Mol Med 1996, 2(5): 637-46.

102. Chong, T., McMillan, M., Teo, J.L., Henderson, W.R. Jr., Kahn, M. *Chemogenomic investigation of AP-1 transcriptional regulation of LTC_4 synthase expression*. Lett Drug Design Dis 2004, 1: 35-44.

103. Shaulian, E., Karin, M. *AP-1 as a regulator of cell life and death*. Nat Cell Biol 2002, 4(5): E131-6.

104. Rahman, I. *Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases*. J Biochem Mol Biol 2003, 36(1): 95-109.

105. Mastruzzo, C., Crimi, N., Vancheri, C. *Role of oxidative stress in pulmonary fibrosis*. Monaldi Arch Chest Dis 2002, 57(3-4): 173-6.

106. Demoly, P., Basset-Seguin, N., Chanez, P., Campbell, A.M., Gauthier-Rouviere, C., Godard, P., Michel, F.B., Bousquet, J. *c-fos proto-oncogene expression in bronchial biopsies of asthmatics.* Am J Resp Cell Mol Biol 1992, 7(2): 128-33.

107. Eynott, P.R., Nath, P., Leung, S.Y., Adcock, I.M., Bennett, B.L., Chung, K.F. *Allergen-induced inflammation and airway epithelial and smooth muscle cell proliferation: Role of Jun N-terminal kinase*. Br J Pharmacol 2003, 140(8): 1373-80.