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Airway Inflammation and Hypersensitivity Induced by Chronic Smoking

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Abstract

Airway hypersensitivity, characterized by enhanced excitability of airway sensory nerves, is a prominent pathophysiological feature in patients with airway inflammatory diseases. Although the underlying pathogenic mechanism is not fully understood, chronic airway inflammation is believed to be primarily responsible. Cigarette smoking is known to cause chronic airway inflammation, accompanied by airway hyperresponsiveness. Experimental evidence indicates that enhanced excitability of vagal bronchopulmonary sensory nerves and increased tachykinin synthesis in these nerves resulting from chronic inflammation are important contributing factors to the airway hyperresponsiveness. Multiple inflammatory mediators released from various types of structural and inflammatory cells are involved in the smoking-induced airway inflammation, which is mainly regulated by redox-sensitive signaling pathways and transcription factors. Furthermore, recent studies have reported potent sensitizing and stimulatory effects of these inflammatory mediators such as prostanoids and reactive oxygen species on these sensory nerves. In summary, these studies using cigarette smoking as an experimental approach have identified certain potentially important cell signaling pathways and underlying mechanisms of the airway hypersensitivity induced by chronic airway inflammation.

Keywords

cigarette smoking; airway inflammation; airway sensory nerves; airway hypersensitivity; cough; COPD

1. Introduction

Airway hypersensitivity, characterized by exaggerated sensory and reflexogenic responses to inhaled irritants, is a common pathophysiological feature in patients with airway inflammatory diseases such as asthma and bronchitis (Widdicombe, 2003; Lee and Gu, 2009). Increasing evidence reported in recent studies suggests that vagal bronchopulmonary

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sensory nerves play an important role in the manifestation of various symptoms of airway hypersensitivity in these patients (Coleridge and Coleridge, 1984; Spina, 1996; Geppetti et al., 2006; Lee, 2009; Lee and Gu, 2009). Activation of bronchopulmonary non-myelinated (C-fiber) sensory endings, the dominant type of afferent fibers in vagus nerves, and a small subset of rapidly adapting receptors is known to elicit centrally mediated reflex responses such as reflex bronchoconstriction and airway hypersecretion, and also evokes airway irritation, cough and dyspneic sensation, (Widdicombe, 1981; Coleridge and Coleridge, 1984; Lee and Pissarri, 2001; Canning et al., 2004; Lee, 2009). Furthermore, it is well documented that the sensitivity of these airway afferents can be enhanced by acute or chronic inflammation or injury of airway mucosa (Lee et al., 2002; Lee and Undem, 2005). Thus, when the excitability of these afferents is enhanced, a given level of bronchoactive challenge will generate a greater intensity of afferent discharge which consequently causes a more severe bronchoconstriction mediated through both the cholinergic reflex pathway and local axon reflexes; the latter involves the release of certain neuropeptides including tachykinins and calcitonin gene-related peptide (CGRP) from the sensory terminals of C-fiber afferents. These neuropeptides are known to act on a number of effector cells in the respiratory tract (e.g., airway and vascular smooth muscles, cholinergic ganglia, various inflammatory cells, and mucous glands, etc.) and produce potent local effects such as bronchoconstriction, extravasation of macromolecules, and edema of airway mucosa (Barnes and Lundberg, 1991; Joos et al., 2000; Barnes, 2001). Intense and/or sustained stimulation of these afferents may therefore lead to neurogenic inflammation in the airways (Barnes, 2001; Groneberg et al., 2004). Taken together, hypersensitivity of these sensory nerves is likely to play an important part in the development of airway hyperresponsiveness induced by inflammation.

It is well documented that cigarette smoking is the major cause of chronic airway inflammatory diseases in humans. Indeed, airway hyperresponsiveness, chronic cough and hypersecretion of mucus are common symptoms of various airway diseases found in human smokers (Gerrard et al., 1980; Chilmonczyk et al., 1993; Braman, 2006). Recent studies indicated the critical involvement of airway sensory nerves in the various abnormal pulmonary functions associated with chronic smoking. Hence, in this mini review we intend to focus specifically on the role of airway sensory nerves in the airway hyperresponsiveness caused by chronic smoking, and the cellular mechanisms underlying the development of smoking-induced airway inflammation and hypersensitivity of these sensory nerves. Chronic exposure to cigarette smoke and airway inflammation are also known to lead to progressive changes in other cell functions and tissue structures in the lung (Thorley and Tetley, 2007; Taylor 2010), which will not be discussed in this review.

2. Airway hyperresponsiveness induced by chronic smoking

Human Smokers

A high prevalence of bronchial hyperresponsiveness in human smokers has been extensively documented in the literature (Gerrard et al., 1980; Mullen et al., 1986; Chilmonczyk et al., 1993; Blanc et al., 2009). Although the pathogenic mechanisms involved are not fully understood, it is believed that the augmented sensitivity of airway afferents associated with chronic smoking-induced airway inflammation and mucosal injury plays a key role in the manifestation of these symptoms (Mullen et al., 1986; Chilmonczyk et al., 1993; Wu and Lee, 1999). For example, an increase in the cough sensitivity to citric acid aerosol has been reported in chronic smokers with chronic obstructive pulmonary diseases (COPD) (Wong and Morice, 1999). A recent study further demonstrated that chronic cigarette smoking is associated with an increase in cough sensitivity to capsaicin aerosol challenge in asymptomatic smokers with normal pulmonary function (Blanc et al., 2009). These

investigators suggested that the increase in airway responsiveness to capsaicin inhalation challenge in cigarette smokers may be related to the degree of inflammatory response.

Animal Studies

Experimental evidence of bronchial hyperresponsiveness induced by prolonged exposure of airways to cigarette smoke has been extensively documented in various animal models, accompanied by airway epithelial injury and mucosal inflammation (Daffonchio et al., 1993; Aguayo, 1994; Wu and Lee, 1999; Kwong et al., 2001). Previous investigators have described in details the extent of injury of the airway mucosa and the subsequent inflammatory reactions caused by repeated exposure to cigarette smoke, which include increased airway vascular permeability, mucosal edema, and migration of polymorphonuclear leukocytes and macrophages into the epithelium (Hulbert et al., 1985; Daffonchio et al., 1993).

Accompanying the airway inflammation was bronchial hyperresponsiveness to the challenges of a variety of bronchoactive substances including acetylcholine, neurokinin A, and capsaicin (Figs. 1 and 2) (Wu and Lee, 1999; Kwong et al., 2001). Furthermore, the cigarette smoke-induced bronchial hyperresponsiveness to other bronchoactive substances (e.g., histamine, substance P, etc.) has also been extensively reported (Hulbert et al., 1985; Dusser et al., 1989a; Daffonchio et al., 1993; Hernandez et al., 1994; Matsumoto et al., 1996). The finding that the bronchial hyperresponsiveness in the chronic-smoking guinea pigs was completely abolished after pretreatment with antagonists to NK₁ (neurokinin type 1)- and NK₂-receptors, indicating the critical involvement of tachykinins in these augmented responses (Fig. 1). Indeed, substance P- and CGRP-like immunoreactivities in the airway tissue were significantly greater in the animals chronically exposed to cigarette smoke than that in the control animals (Fig. 3A). Furthermore, β -preprotachykinin (β -PPT, a splice variant from the PPT A gene encoding tachykinins including SP and NKA) mRNA levels as measured by *in situ* hybridization histochemistry showed a significant increase in vagal jugular ganglion neurons (Fig. 3B). Collectively, these findings suggested that an increase in tachykinin synthesis and release in jugular ganglion neurons innervating the lungs and airways is a major contributor to the chronic cigarette smoke-induced airway hyperresponsiveness (Wu and Lee, 1999; Kwong et al., 2001). Further, these results are in general agreement with the observations that chronic airway inflammation induces a substantial increase in the synthesis of tachykinins; for example, the level of PPT A mRNA expression was significantly elevated in the nodose ganglia after airway inflammation was induced by chronic exposure to ovalbumin (Fischer et al., 1996).

The observation that the bronchoconstrictive response to the challenges of exogenous tachykinin was also augmented in the chronic-smoking animals (Fig. 2) further suggested that some additional factors were probably also involved. Previous investigators have reported that the tachykinin-receptor mRNA expression is higher in the airways of human smokers than in those of nonsmokers, suggesting that chronic airway inflammation may have induced such increases in tachykinin-receptor gene expression (Bai et al., 1995). An increase in the densities of tachykinin receptors in the airway smooth muscle may then lead to an augmented response to a given level of endogenously released or exogenously administered tachykinins.

It has been shown that airway inflammation inhibits the enzyme activity of neutral endopeptidase (NEP) that is present on the membranes of various cell types (including epithelium and nerve fibers) in the airways, and can cleave tachykinins immediately after their release (Dusser et al., 1989b; Hsiue et al., 1992). In addition, various components of cigarette smoke are also known to depress the activity of NEP (Dusser et al., 1989a). The inhibition of NEP activity induced by cigarette smoke has been suggested to be due to

oxidative stress-inactivation of the NEP enzyme through chemical reaction and promotion of NEP protein degradation via lysosomal and/or proteasomal pathways (Wick et al., 2011). Hence, it seems plausible that chronic smoking-induced airway hyperresponsiveness may have resulted from the attenuated NEP activity and consequently the augmented bronchoconstrictive effect of endogenous tachykinins.

Some of the most prominent pathophysiological features associated with chronic smoking in both humans and experimental animals are chronic cough and airway irritation, indicating an involvement of hypersensitivity of the bronchopulmonary afferents. Recent studies have further revealed certain cell signaling mechanisms possibly involved in the development of airway inflammation and hypersensitivity.

3. Cellular mechanisms of airway inflammation induced by chronic smoking

Airway inflammation in chronic smokers

Chronic smoking-induced persistent airway inflammation results from the detrimental effects from multiple inflammatory mediators released from various types of structural cells (e.g., airway epithelial and smooth muscle cells) and inflammatory cells (e.g., macrophages, neutrophils and lymphocytes) (Barnes, 2004; Roth, 2008), and is a major clinical characteristic of patients with COPD (Chung and Adcock, 2008; Roth, 2008). The major types of inflammatory mediators involved in this airway disease include cytokines [e.g., tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β) and IL-6], chemokines (e.g., IL-8, growth-related oncogene- α and macrophage inflammatory protein-1) and lipid mediators (e.g., prostaglandin E₂ (PGE₂) and leukotriene B₄) (Barnes, 2004; Roth, 2008). Clinical studies have revealed that airway levels of these inflammatory mediators are elevated in chronic smokers with COPD (Keatings et al., 1996; Traves et al., 2002; Montuschi et al., 2003).

The complexity of the pathogenetic mechanisms of the cigarette smoke-induced airway inflammation is increased by repeated airway exposure to cigarette smoke in chronic smokers. For each insult, cigarette smoke may directly stimulate airway epithelial cells to increase the production and release of inflammatory mediators that initiate airway inflammation. Additionally, chemokines and other chemotactic mediators released from airway epithelial cells promote the recruitment of inflammatory cells into the airways (Thorley and Tetley, 2007), which would further release additional inflammatory mediators, thereby attracting more inflammatory cells to the inflamed sites. This cycle sustains the progression of airway inflammation, which is amplified in chronic smokers whose airways receive repeated cigarette smoke exposure.

Lung oxidative stress and Cigarette Smoke-induced airway inflammation

The lung of chronic smokers displays an increase in oxidative stress, partly due to excess production of reactive oxygen species (ROS) (Rahman and Adcock, 2006). The major types of ROS are superoxide anion, hydrogen peroxide and hydroxyl radical, all of which may be generated exogenously from the oxidants in the cigarette smoke or endogenously by the metabolic reaction within the cell (Pryor, 1992; Rahman and Adcock, 2006). The increased oxidative stress in the airways of chronic smokers is known to play an important pathophysiological role in the disease by amplifying the airway inflammation (Barnes, 2004; Rahman and Adcock, 2006).

Cigarette smoke contains ROS and many radical precursors that may continuously generate ROS upon reaching the airway tissues (Pryor, 1992). Additionally, infiltrating inflammatory

cells are able to produce and release ROS into bronchoalveolar lavage fluid and airway tissues in chronic smokers (Morrison et al., 1999; Bowler et al., 2004; MacNee, 2005). These extracellular ROS may produce oxidation of membrane phospholipids resulting in generation of several lipid peroxidation products. It is believed that the harmful impact of cigarette smoke on the airways is mainly due to the effect of chemical constituents in the smoke (Pryor, 1992). For this reason, cigarette smoke extract (CSE) has been widely used to study the cellular and molecular mechanisms underlying the lung inflammatory induced by cigarette smoke using cell models. Several in vitro studies have shown that CSE can increase intracellular ROS in airway structural cells (Rahman and Adcock, 2006; Cheng et al., 2009; Faux et al., 2009; Hsu et al., 2009; van der Toorn et al., 2009). These cigarette smoke-mediated increases in ROS, either directly (Rahman and Adcock, 2006; Cheng et al., 2009; Faux et al., 2009; Lin et al., 2010a) or via the formation of lipid peroxidation products (Parola et al., 1999; Uchida et al., 1999; Rahman and Adcock, 2006), may activate signaling pathways and transcription factors resulting in increases in the production of inflammatory mediators in various types of cells.

Several cellular organelles, such as mitochondria and peroxisomes, are major compartments for the generation of intracellular ROS (Kowaltowski et al., 2009; Antonenkov et al., 2010). A recent study (van der Toorn et al., 2009) reported that the lipophilic components present in cigarette smoke can enter airway epithelial cells and increase intracellular ROS production by disturbing mitochondrial function. In mammalian cells, several enzyme systems including NADPH oxidase and peroxisomes may contribute to the generation of intracellular ROS (Borges et al., 2002; Bedard and Krause, 2007).

Role of NADPH oxidase in cigarette smoke-induced airway inflammation

Among the cellular enzyme systems for ROS generation, NADPH oxidase has recently received much attention in the research area of cigarette smoke-induced airway inflammation because it is a membrane-bound enzyme complex that can readily react to the cigarette smoke insult (Yao et al., 2007; Tollefson et al., 2010; Cheng et al., 2011). NADPH oxidase is composed of five subunits and, upon stimulation by cigarette smoke, the three cytosolic components (p40^{phox}, p47^{phox} and p67^{phox}) are recruited to the plasma membrane and bind with the two membrane components (p22^{phox} and gp91^{phox}) to form the active complex (Yao et al., 2007; Tollefson et al., 2010). Activated NADPH oxidase can transport electrons across the plasma membrane to reduce oxygen to superoxide anion, which quickly reacts to generate other ROS (Bedard and Krause, 2007).

Several recent studies have demonstrated that activation of NADPH oxidase by CSE leads to increases in expressions of cytosolic phospholipase A2 (cPLA2) and cyclooxygenase-2 (COX-2) (Fig. 4) as well as the production of their enzymatic product PGE₂ in airway smooth muscle cells (Cheng et al., 2009; Cheng et al., 2010; Lin et al., 2010a). In these airway structure cells, CSE sequentially causes increases in translocation of p47^{phox} to the plasma membrane (Fig. 4H), activity of NADPH oxidase (Fig. 4E), intracellular ROS (Fig. 4C), and expression of cPLA2 and COX-2 (Fig. 4A and 4B). Importantly, the CSE-induced increases in intracellular ROS and expression of both cPLA2 and COX-2 (Fig. 4) can be largely attenuated by pretreatment with a ROS scavenger, an inhibitor of NADPH oxidase or p47^{phox} siRNA for gene knockdown (Fig. 4). Since PGE₂ is a well-known inflammatory mediator, these findings suggest that NADPH oxidase and the intracellular ROS derived from this enzyme system may play a vital role in the cigarette smoke-induced airway inflammation (Lin et al., 2010a).

The mechanism by which cigarette smoke activates NADPH oxidase is not totally clear. In airway smooth muscle cells (Cheng et al., 2010; Lin et al., 2010a), CSE promotes phosphorylation of c-Src at Tyr-416 (Fig. 5D), which is critical for triggering several

sequential events (Fig. 6) including translocation of p47^{phox} to the plasma membrane (Fig. 5G and 5H), activation of NADPH oxidase (Fig. 5F), increase in intracellular ROS (Fig. 5E) and induction of COX-2 (Fig. 5A and 5B). Additionally, the activation of NADPH oxidase by CSE requires the formation of a complex encompassing c-Src, p47^{phox} and NADPH oxidase (Fig. 6), suggesting possible protein-protein interactions (Cheng et al., 2010; Lin et al., 2010a). Interestingly, these molecular events essential for the activation of NADPH oxidase by CSE appear to depend on the activation of the toll-like receptor 4 (TLR4)-MyD88-TRAF6 signaling (Fig. 6) (Lin et al., 2010a). Thus, these findings may partly explain the observations that TLR4 contributes to the induction of airway inflammation by cigarette smoke in murines (Maes et al., 2006; Doz et al., 2008) and cytokine production by CSE in human macrophages (Karimi et al., 2006), both of which are independent from the effect of lipopolysaccharide.

Signaling pathways and transcription factors for induction of cigarette smoke-induced airway inflammation

The cigarette smoke-induced airway inflammation is regulated by redox-sensitive signaling pathways and transcription factors (Rahman and MacNee, 2000). The mitogen-activated protein kinase (MAPK) family is the most extensively studied signaling pathways for induction of cigarette smoke-induced airway inflammation (Mossman et al., 2006). MAPKs are sequentially activated within a cascade of protein kinases and subdivided into three major pathways: extracellular signal-regulated kinase (ERK), c-Jun-NH2-terminal kinase (JNK) and p38 kinase (Mossman et al., 2006). Several *in vitro* studies using airway structural cells or inflammatory cells have demonstrated that CSE can activate one or more MAPK pathways leading to increased production of inflammatory mediators (Hellermann et al., 2002; Mossman et al., 2006; Li et al., 2007; Winkler et al., 2008; Cheng et al., 2009; Lin et al., 2010a; Cheng et al., 2011). The activation of MAPK pathways by CSE appears to be mediated through the increase in ROS (Rahman and MacNee, 2000; Mossman et al., 2006; Rahman and Adcock, 2006), likely resulting from the activation of NADPH oxidase (Yao et al., 2007; Cheng et al., 2009; Lin et al., 2010a). Other oxidant-sensitive pathways such as PI3K/Akt (Yang et al., 2009) or protein kinase C signaling (Chung and Adcock, 2008; Yao et al., 2010) may also involve in the induction of airway inflammation by cigarette smoke.

Activator protein 1 (c-Fos/c-Jun, AP-1) and nuclear factor- κ B (NF- κ B) are two redox-sensitive transcription factors that are crucial for induction of cigarette smoke-induced airway inflammation in animal models (Marwick et al., 2004; Rahman and Adcock, 2006; Yao et al., 2007; Li et al., 2009) and CSE-induced increase in production of inflammatory mediators in airway structure cells and inflammatory cells (Mossman et al., 2006; Yao et al., 2007; Cheng et al., 2009; Murugan and Peck, 2009; Cheng et al., 2011). AP-1 exists as either c-Jun/c-Jun (a homodimer complex) or c-Fos/c-Jun (a heterodimer complex), which is activated by the phosphorylation of JNK and other MAPKs leading to its nuclear translocation (Mossman et al., 2006). NF- κ B usually exists as a heterodimeric complex of p50 and p65/RelA subunits. In the cytoplasm, NF- κ B is inactive due to its association with I κ B, an inhibitor protein that masks the nuclear translocation signal within NF- κ B. Upon cell stimulation with oxidants such as cigarette smoke, I κ B is rapidly phosphorylated, which promotes the ubiquitination process and subsequent degradation of this inhibitor protein by the proteasome (Rahman and Adcock, 2006). The activation of NF- κ B may or may not depend on the activation of MAPK pathways (Mossman et al., 2006; Cheng et al., 2009). After dissociation with I κ B, the NF- κ B heterodimer becomes an active form for nuclear translocation. Subsequently, the nuclear AP-1 and NF- κ B can then activate their target genes by binding with their corresponding DNA elements within promoter regions leading to increased expressions of inflammatory genes (Mossman et al., 2006; Rahman and Adcock, 2006). This AP-1- or NF- κ B-dependent increase in inflammatory gene transcription may

also be regulated by the process of histone acetylation via coactivators such as CREB-binding protein and p300 (Rahman and Adcock, 2006; Yang et al., 2009; Cheng et al., 2011).

4. Sensitizing effects of inflammation on airway afferents

Increased syntheses and releases of certain autacoids and cytokines resulting from the chronic smoking-induced airway inflammatory reactions described above are known to lead to airway hypersensitivity. In experimental animals (e.g., guinea pigs), chronic exposure of the airways to cigarette smoke induced cough hypersensitivity to various tussive inhalation challenges (Karlsson et al., 1991; Bergren, 2001). The enhanced responsiveness gradually returned to normal over a period of three weeks. Chronic smoking also elevated the cough sensitivities to aerosolized capsaicin and bradykinin, suggesting an involvement of capsaicin-sensitive sensory nerves innervating the airways (Bergren, 2001). The sensitizing effects generated by some of these inflammatory mediators on airway afferents are believed to play a critical role.

Prostanoids

Elevated levels of endogenous prostanoids and their metabolites found in the bronchoalveolar lavage fluid and other body fluids in human smokers and experimental animals exposed to cigarette smoke have been extensively documented (Hulbert et al., 1985; Aguayo, 1994; Hernandez et al., 1994; Matsumoto et al., 1996). It is known that thromboxane A₂, PGE₂ and PGI₂ are involved in the sustaining irritant effect on the respiratory tract and bronchoconstriction evoked by acute or chronic inhalation of cigarette smoke. In addition to the direct pharmacological effects on the various target cells in the lung, these autacoids have been shown to exert profound modulatory effects on the physiological functions of sensory receptors, cholinergic neurons, and smooth muscles in the airways. PGE₂, a potent autacoid derived from arachidonic acid metabolism through the enzymatic action of COX-2 and PGE synthase, has been considered as one of the primary candidates, and therefore its signaling mechanisms are discussed in more details here. The airway epithelium, which is the main target of initial assault by the inhaled cigarette smoke, is also the major cellular source of this autacoid (Holtzman, 1991). Inhaled PGE₂ enhances the sensitivity of the cough reflex elicited by capsaicin in humans (Choudry et al., 1989), suggesting a PGE₂-induced sensitization of pulmonary C-fiber afferents. Indeed, this contention is supported by a recent observation; exogenous PGE₂ at a low dose does not cause any systemic effects (without significant changes in baseline cardiovascular parameters), but markedly enhances the excitabilities of pulmonary C fibers to chemical stimulants (e.g., Fig. 7A) and to lung inflation in anesthetized rats (Ho et al., 2000). In cultured vagal (nodose and jugular ganglia) pulmonary sensory neurons identified by retrograde labeling with fluorescent tracer and neuronal activity measured by the perforated patch-clamp technique, PGE₂ (1 μM) markedly increased the whole cell current density and the number of action potentials evoked by either current injection or chemical stimulants (e.g., Fig. 7B) (Kwong and Lee, 2002). PGE₂ increased the magnitude of peak current and caused a hyperpolarizing shift of the current-voltage activation curve obtained by depolarizing voltage steps (Kwong and Lee, 2002). The sensitizing effect of PGE₂ could also be mimicked by butaprost (Kwong and Lee, 2002), a selective agonist of the EP₂ prostanoid receptor, or by a direct activation of adenylyl cyclase or protein kinase A (PKA), and prevented by pretreatment with inhibitors of adenylyl cyclase or PKA (Gu et al., 2003). Taken together, these studies suggest that PGE₂ activates the G_s protein-coupled EP₂ prostanoid receptor present on the membrane of C neurons, which upon activation increases the enzyme activity of adenylyl cyclase (Cui and Nicol, 1995; England et al., 1996; Lee et al., 2002; Gu et al., 2003). Presumably, the resulting rise in cAMP may then stimulate PKA, which in turn enhances the neuronal excitability by increasing the phosphorylation of both

ligand-gated channels and voltage-sensitive channels; the former includes the TRPV1 and 5-HT₃ receptors (Kwong and Lee, 2002), and the latter includes the TTX-R I_{Na} (Kwong and Lee, 2005). Based upon the studies in DRG nociceptive C neurons, other voltage-sensitive currents that may be involved in the PGE₂-induced airway hypersensitivity include a delayed rectifier-like outward potassium current (Evans et al., 1999) and a calcium-dependent potassium current, I_{sAHP} (Fowler et al., 1985; Gold et al., 1996).

Reactive oxygen species (ROS)

As discussed earlier, cigarette smoke not only contains a large quantity of ROS, but also triggers the endogenous production of ROS (Pryor, 1992; Rahman and Adcock, 2006). An earlier study has reported that inhalation of gas phase cigarette smoke elicited bradypnea and apnea in anesthetized rats, which was mediated through an activation of vagal bronchopulmonary C-fiber afferents, and hydroxyl radicals either released endogenously in the lung or delivered by smoke were probably involved because the effect was prevented by a pretreatment with dimethylthiourea, a hydroxyl radical scavenger (Lee, 1990). Recent studies have further demonstrated the stimulatory effect of cigarette smoke on bronchopulmonary C-fiber afferents in rats, and the effect is partially mediated through an activation of the TRPA1 channels by ROS (Lin et al., 2010b); TRPA1, a member of the transient receptor potential (TRP) family of ion channels, is known to be expressed in a subset of vagal bronchopulmonary C-fibers (Nassenstein et al., 2008; Taylor-Clark et al., 2008). Indeed, TRPA1 may function as a transducer in detecting ROS in the airways and mediating its irritant effects and cardiopulmonary reflex responses (Bessac et al., 2008; Sawada et al., 2008; Taylor-Clark and Udem, 2010). Whether the effect on TRPA1 is generated by a direct action of ROS or through a secondary effect involving release of intermediate molecules or inflammatory mediators is not yet known. The role of TRPA1 in the stimulatory effects of ROS on airway afferents is reviewed in more details by Udem and co-workers in this issue (2011). Furthermore, other investigators have recently reported that activation of the TRPV1 receptor, another TRP channel and a polymodal transducer selectively expressed on C-fiber sensory nerves, by cyclooxygenase metabolites is partially responsible for ROS-evoked stimulation of pulmonary C-fiber afferents in rats because the stimulatory effect of aerosolized hydrogen peroxide was reduced to half by pretreatment with either capsazepine, a selective TRPV1 antagonist, or indomethacin, a COX inhibitor (Ruan et al., 2005, 2006).

Other mediators

Several other autacoids have been shown to increase their productions in the respiratory tract during chronic smoking-induced airway inflammation, and also known for their sensitizing effects on bronchopulmonary C-fiber afferents. For example, a high level of TNF- α has been detected in the serum of human smokers in a cigarette dose-dependent manner (Petrescu et al., 2010), supporting the suggested importance of TNF- α in the pathogenesis of cigarette smoke-induced chronic pulmonary diseases (Wright et al., 2007). Interestingly, recent studies have shown that pretreatment with TNF- α induced a pronounced and sustained potentiating effect on the pulmonary chemoreflex responses in anesthetized mice (Lin and Lee, 2009) and the sensitivity to TRPV1 activators in isolated pulmonary sensory neurons (Hu et al., 2010), suggesting that this pro-inflammatory cytokine may contribute in part to the airway hyperresponsiveness induced by chronic smoking. Further, it has been reported that the plasma leukotrienes C₄, D₄, and E₄ are increased after inhalation of cigarette smoke in healthy volunteers (Kobayashi et al., 1988) and that blood obtained from smokers produces more leukotriene E₄ in response to calcium ionophore A23187 than the blood from non-smokers (Riutta et al., 1995). In addition, histamine, a classical bronchoconstrictive inflammatory mediator, is released primarily from mast cells, and mast cells are known to increase in number and to change in phenotype within the lungs of smokers (van Overveld,

1989; van Overveld et al., 1989). The sensitizing effect of histamine on the vagal bronchopulmonary C-fiber endings has been clearly demonstrated in anesthetized dogs (Lee and Morton, 1993).

5. Summary and Conclusion

Although the underlying mechanisms of the airway hyperresponsiveness generated by chronic smoking are still not fully understood, in this review we have presented evidence illustrating that enhanced excitability of vagal bronchopulmonary sensory nerves and increased tachykinin synthesis in these nerves are probably important contributing factors. Recent studies have further uncovered certain cell signaling pathways involved in the development of airway inflammation caused by chronic smoking. As the result, endogenously released inflammatory mediators, such as prostanoids and ROS, exert potent stimulatory and sensitizing effects on these sensory nerves, which leads to airway hypersensitivity.

Despite all these findings, many important questions concerning the development of airway hypersensitivity during chronic airway inflammation, such as that resulting from chronic smoking, still remain. For example, are the same signaling pathways and transcription factors involved in other airway structural and inflammatory cells (e.g., epithelial cells, macrophages, etc.) exposed to cigarette smoke constituents as in the smooth muscle cells illustrated in the studies described here? To what extent do the excessive production of ROS and over expression of COX-2 and cPLA and their enzymatic products contribute to the plasticity of airway sensory nerves as well as the remodeling and repair of other airway structures (e.g., airway smooth muscles) at cellular, subcellular and molecular levels during the progressive development of chronic airway diseases (e.g., chronic bronchitis) as the result of chronic smoking? Another interesting question is whether the expression of receptor proteins (e.g., prostanoid EP receptors, TRPA1 and TRPV1 channels, etc.) on the target cells (e.g., sensory terminals) is altered by the repetitive challenges during chronic exposure to smoke. Answers to some of these questions will undoubtedly provide a more in-depth understanding of the etiology of smoking-related airway diseases, and possibly lead to the development of more effective therapeutic and preventive strategies.

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Figure 1.

Experimental record illustrating the responses of transpulmonary pressure (P_{tp}), respiratory flow ($\dot{V}$; inspiratory flow: positive deflection), and arterial blood pressure (ABP) to capsaicin before and 30 min after administration of CP-99994 and SR-48968, selective antagonists of NK₁ and NK₂ receptors, respectively, in a control (425 g) and a cigarette smoke (CS)-exposed (415 g) guinea pig. *Arrows* indicate times of bolus injection of capsaicin (Cap; 1.68 μ g/kg) into the venous catheter (from Kwong et al., 2001).

Figure 2.

Dose responses of total pulmonary resistance (R_L) and dynamic lung compliance (C_{dyn}) to intravenous injections of ACh (A), NKA (B), and Cap (C) in control and cigarette smoke (CS)-exposed guinea pigs. Values are means $\pm$ SE of 8 guinea pigs in each group. Peak response to each dose of ACh in each animal was averaged over 3 consecutive breaths that occurred within 10 breaths after each injection. Peak response to each dose of NKA or Cap was averaged over 6 consecutive breaths that occurred within 30 breaths after each injection. * Significant difference comparing corresponding data between control and CS animals, $P < 0.05$ (from Wu and Lee, 1999).

Figure 3.

A. Effect of cigarette smoke (CS) inhalation on SP-LI and CGRP-LI in bronchial tissue from control (*open bars*) and CS-exposed (*filled bars*) animals. SP-LI and CGRP-LI were measured by RIA and expressed in femtomoles per gram of tissue. *Significant difference between control and CS-exposed animals. Data represent means $\pm$ SEM of 8 guinea pigs in each group. **B.** Enhanced expression of β -PPT mRNA per cell in dorsal root, jugular, and nodose ganglia. *In situ* hybridization histochemistry was quantified by measuring the area covered by silver grains per cell. *Open bars*, tissue harvested from control animals; *filled bars*, tissue from CS-exposed animals. * Significant difference from control animals. Data

are expressed as means $\pm$ SEM with 10 guinea pigs in each group except for the nodose ganglia of the CS group ($n = 9$) (modified from Kwong et al., 2001).

Figure 4.

Cigarette smoke extract (CSE)-induced COX-2 expression is mediated through NADPH oxidase-dependant ROS generation in human airway smooth muscle cells. (A) Cells were pretreated with NAC (N-acetylcysteine; a ROS scavenger), DPI (diphenyleneiodonium chloride; an inhibitor of NADPH oxidase), or APO (apocynin; an inhibitor of NADPH oxidase) prior to CSE stimulation for 24 h. (B) Cells were pretreated with NAC, DPI, or APO prior to CSE stimulation for 6 h. (C) Cells were labeled with DCF-DA prior to CSE stimulation. (D) Cells were pretreated with NAC, DPI, or APO or were incubated with LPS-RS (*Rhodobacter sphaeroides* LPS; a potent antagonist of LPS) prior to CSE stimulation for 1 h. (E, F) Cells were stimulated with CSE for the indicated times or pretreated with DPI or APO prior to CSE stimulation for 10 min. (G) Cells were transfected with scrambled siRNA or p47^{phox} siRNA prior to CSE stimulation for 24 h. (H) Cells were stimulated with CSE for the indicated times or pretreated with DPI or APO prior to CSE stimulation for 10 min. The membrane and cytosolic levels of p47^{phox} were analyzed. In panels A, G and H, protein levels were examined by Western blot. In panel B, mRNA levels were analyzed by real-time PCR. Data are expressed as means ± SEM ($n = 4$ in each group). * $P < 0.05$, # $P < 0.01$

compared with the cells exposed to CSE alone (A, B, D, F, G). * $P < 0.05$, # $P < 0.01$ compared with the basal level (C, E) (from Lin et al., 2010a).

Figure 5.

Cigarette smoke extract (CSE)-induced COX-2 expression is mediated through c-Src in human airway smooth muscle cells. (A) Cells were pretreated with PP1 (an inhibitor of c-Src) prior to CSE stimulation for 24 h. (B) Cells were pretreated with PP1 prior to CSE stimulation for 6 h. (C) Cells were transfected with scrambled siRNA or Src siRNA prior to CSE stimulation for 24 h. (D) Cells were pretreated without or with PP1 prior to CSE stimulation for the indicated times. (E) Cells were labeled with DCF-DA and then pretreated with PP1 prior to CSE stimulation for 1 h. (F) Cells were pretreated with PP1 prior to CSE stimulation for 10 min. (G) Cells were pretreated with PP1 prior to CSE stimulation for 10 min. The membrane and cytosolic levels of p47^{phox} were analyzed. (H) Cells were pretreated without or with PP1 prior to CSE stimulation for 10 min. The immunoprecipitation used an anti-c-Src antibody and the immunoprecipitates were then analyzed using an anti-p47^{phox} or anti-c-Src antibody. In panels A, C, D, G and H, protein levels were examined by Western blot. In panel B, mRNA levels were analyzed by real-time PCR. Data are expressed as means ± SEM ($n = 4$ in each group). * $P < 0.05$; # $P < 0.01$ compared with the cells exposed to CSE alone (from Lin et al., 2010a).

Figure 6.

Schematic diagram illustrating the proposed signaling pathway involved in cigarette smoke extract-induced increase in expressions of inflammatory genes in airway smooth muscle cells. Cigarette smoke extract activates the TLR4/MyD88/TRAF6 signaling leading to the formation of a complex encompassing c-Src, p47^{phox} and NADPH oxidase. The activated NADPH oxidase then promotes generation of reactive oxygen species (ROS), which in turn initiates the activation of redox-sensitive signaling pathways (e.g., MAPKs and PI3K/Akt) and transcription factors (e.g., AP-1 and NF-κB) and ultimately induces the increase in expressions of various inflammatory genes (modified from Cheng et al., 2010; Lin et al., 2010a).

Figure 7.

A: Experimental records illustrating effect of PGE₂ on the responses of pulmonary C fibers to right-atrial injections of chemical stimulants in 4 different rats anesthetized and artificially ventilated. Upper panels, control responses; lower panels, responses during a constant infusion of PGE₂ (1.5–3.0 $\mu\text{g/kg/min}$ for 2 min, iv). Panels from left to right: 1) responses to capsaicin (Cap; 0.5 $\mu\text{g/kg}$); receptor location, right upper lobe; body weight, 392 g. 2) anandamide (Ana; 0.4 mg/kg); right middle lobe; 410 g. 3) phenylbiguanide (PBG; 4 $\mu\text{g/kg}$); right middle lobe; 430 g. 4) lactic acid (LA; 9 mg/kg); right middle lobe; 425 g. **B:** Experimental records recorded in current clamp mode illustrating capsaicin-evoked depolarization and action potentials in a small nodose neuron (28.1 pF) in the absence and presence of PGE₂ (10^{-6} M). Bar: capsaicin (10^{-7} M) application. Panel a, before (control; resting potential = -56 mV); b, during PGE₂ perfusion; c, 60 min after removal of PGE₂ (recovery); and d, during the second perfusion with PGE₂ of the same concentration (modified from Lee et al., 2002).