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The Effect of Cigarette Smoke-derived Oxidants on the Inflammatory Response of the Lung

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Abstract

The inhalation of cigarette smoke triggers a marked cellular influx in the lung and this inflammation is believed to play a central role in the development of smoke-related lung diseases such as asthma and COPD. Studies demonstrate that smoke-derived oxidants are a major factor in this inflammatory reaction to cigarette smoke. These oxidants can overwhelm the lung's antioxidant defenses and they can up regulate inflammation by a number of mechanisms. Free radicals directly stimulate the production of chemotactic compounds such as 8-isoprostane. In addition, smoke-derived oxidants can activate several intracellular signaling cascades including NF- κ B, MAPK and AP-1. This transcriptional activation induces the expression of cytokines and intracellular adhesion molecules that facilitates the trafficking of neutrophils, macrophages and lymphocytes into the lung. Moreover, oxidants can promote chromatin remodeling that facilitates the expression of proinflammatory genes by stimulating the acetylation of histone residues in the nucleosome. This leads to conformational changes that enhance expression by rendering the gene more accessible to binding to transcriptional factors. Thus, the oxidant-antioxidant imbalance generated by cigarette smoke can promote inflammation which is critical to the functional decline that occurs in both asthma and COPD patients. Future research is needed to better define the effects of smoke-derived oxidants on lung inflammation and to determine the most efficacious strategies for generating significant antioxidant protection in the lung.

Keywords

Inflammation; Emphysema; Asthma; Regulation; Transcription; Induction

Introduction

Cigarette smoking is an important etiologic factor linked to the development of asthma [1, 2] and chronic obstructive pulmonary disease [COPD]. These diseases afflict millions worldwide and are two of the leading causes of death in industrialized societies. While the age adjusted mortality for cardiovascular diseases has dramatically decreased over the past thirty years, it has risen by 60% for asthma [3] and 103% for COPD [4] in the same time interval. In spite of the decline in the rate of smoking, with the ageing of the population, the incidence of this disease has actually increased over the past thirty years [5]. Enhanced insights into the basic mechanisms of these diseases will be required in order to improve these statistics. Both asthma and COPD are characterized by the induction of genes encoding for multiple inflammatory proteins including cytokines, growth factors and adhesion molecules. The result is an influx of inflammatory cells into the lung. These cells

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release powerful mediators that alter bronchial airway reactivity, damage the epithelium and lead to fundamental changes in the architecture of the lung. Cigarette smoke is a potent inflammatory stimulus that is directly linked to the incidence and severity of asthma and COPD. Thus, determining the mechanisms whereby cigarette smoke mediates its proinflammatory effects would provide much needed insights into the pathogenesis of lung diseases.

The high concentration of oxidant molecules in cigarette smoke is believed to play a major role in the development of smoke-related lung diseases. It is estimated that each cigarette puff contains 10^{14} free radicals [6]. While the oxidants in the gas phase are short-lived and affect primarily the upper respiratory tract, the oxidants in the tar phase contain relatively stable semiquinone radicals that may be particularly harmful to the lung itself [7]. These radicals are capable of damaging epithelial cells of the lower respiratory tract through oxidative injury to membrane lipids, proteins, carbohydrates and DNA. The injury can significantly impair cellular function, induce apoptosis and stimulate dysfunctional matrix remodeling [7]. Semiquinone radicals present in the tar phase of cigarettes are less volatile and they can cause sustained production of $O_2^\bullet$, H_2O_2 and $OH^\bullet$ reactive oxygen species [8]. While these free radicals are capable of generating direct oxidative damage to the lung, their ability to induce inflammation may be one of the most pivotal factors in the pathogenesis of COPD and asthma.

Chemical studies of cigarettes have elucidated how these devices form reactive oxygen species. A recent study directly measured the levels of free radicals present in cigarette smoke [9]. The concentration of total reactive oxygen species in three major cigarette brands tested ranged from 16 to 55 nmol H_2O_2 /l [9]. Further analysis demonstrated that the free radicals were generated by the combustion of the tobacco leaves and were not present in the intact leaf or in the ash. The levels of reactive oxygen species correlated well with the level of tar that was present in the cigarette [9]. This data affirms the important contribution that tar makes to the development of smoke-induced oxidative injury. Of note, cigarette filters did not affect the level of free radicals that were detected in mainstream cigarette smoke. In fact, a separate study found that the use of a "bio-filter" actually increased the production of tar phase and stable free radicals [10]. Thus, the use of filter devices has the potential to actually augment the risk to smokers.

Smoke-derived Oxidants and Inflammation

Once formed, free radicals can promote lung inflammation by generating compounds that induce the chemotaxis of cells into the lung. Isoprostanes are prostaglandin-like compounds that are produced independent of the cyclooxygenase pathway by the lipid peroxidation of arachidonic acid. These phospholipids which are present in the cell membrane are oxidized by free radicals and then released by the action of phospholipases. Upon release, isoprostanes can have potent effects on the inflammatory response of the lung. 8-isoprostane was proinflammatory in human macrophages for the induction of IL-8 and macrophage inflammatory protein 1 alpha [11]. Moreover, a recent study demonstrated that isoprostanes can promote the influx of neutrophils by stimulating β_2 -integrin-mediated adhesion of human neutrophils [12]. These results demonstrate that 8-isoprostane can directly amplify the inflammatory responses of the lung. There is significant data to suggest that oxidants present in cigarette smoke can augment the levels of isoprostanes detected in smokers [13]. Morrow and colleagues demonstrated a marked increase in F2-isoprostane in the serum of active smokers [14]. These levels decreased to near baseline upon cessation of smoking. Interestingly, even passive cigarette smoke exposure can increase the level of F2 isoprostane detected in the serum [15]. These results demonstrate that smoke-derived oxidants can elicit

the production of compounds that contribute to the pathophysiology of these smoke-related lung diseases.

Evidence for Oxidative Damage in COPD and Asthma

Numerous studies have shown that free radicals produced by cigarette smoke can lead to the formation of oxidative damage in the lung. Howard et al examined the effects of cigarette smoke on the formation of 8-hydroxy-2'-deoxyguanosine [8-OH-dG], an oxidative DNA product, within the lungs of mice [16]. A single smoke exposure caused a 40% increase in lung 8-OH-dG. This rapid increase demonstrated that even single, occasional, or brief exposures to cigarette smoke can damage lung DNA. Similarly, a study in guinea pigs found that smoke-derived oxidants cause a significant increase in the formation of protein carbonyl and bityrosine as well as loss of tryptophan residues and thiol groups [17]. Several studies have identified markers of free radical damage are present in patients with COPD. Increased levels of urinary 8-OH-dG have been detected in the urine of COPD patients [18] and elevated levels of 3-nitrotyrosine [19] and lung lipid peroxidation products [20] are detected in the airway cells and epithelium of COPD patients. All of these oxidative markers had an inverse correlation with lung function suggesting that they could be key mediators of the physiologic deterioration that is noted in patients with this disease.

An increase in oxidative stress has also been noted in asthmatic patients. Elevated levels of hydrogen peroxide [21] and total nitrite/nitrates [22] were measured in the exhaled breath of asthmatics. Moreover, augmented levels of nitrotyrosine [23] and chlorotyrosine [24] were detected in the lungs of patients with asthma. Airway inflammatory cells are likely responsible for this increase in oxidative stress. Macrophages isolated from the airways of asthmatics produce more superoxide than macrophages from control patients [25] and antigen challenge markedly increases the release of reactive oxygen species by eosinophils from asthmatic patients [26]. The oxidants released by these cells may cause detrimental alterations in the structure and function of the lung. For instance, superoxide reacts with endogenous and exogenous nitric oxide to produce peroxynitrite, a potent free radical that has been shown to increase airway hyperresponsiveness [27]. In addition, the addition of free radicals to epithelial cells stimulates arachadonic acid metabolism and induces the release of mucin from these cells [28]. More recently, free radicals have been shown to promote mucin release in pulmonary epithelial cells by activating the epidermal growth factor receptor thus triggering the MAP Kinase [MAPK] signaling pathway [29]. Together, these studies demonstrate the ability of oxidants to reproduce key pathogenic features present in asthma.

Antioxidants and Smoke-related Lung Diseases

All cells are exposed to oxidative stress during the course of normal metabolism. The primary sites of production are the electron transport chain of the mitochondria and oxidant generating enzymes present in the cell [e.g. xanthine oxidase, nitric oxide synthase and nicotinamide adenine dinucleotide phosphate reduced oxidase]. The lung, given its direct exposure to oxygen in the ambient air, experiences enhanced oxidant stress compared to other organs. However, the lung has a rich network of antioxidants to protect itself from free radical injury. This endogenous network can be divided into enzymatic and non-enzymatic categories. The enzymatic antioxidants include superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and thioredoxin families. The SOD family of enzymes is an exciting area of focus for both asthma and COPD related research. This family of enzymes is comprised of three groups: CuZn SOD [SOD1] which is the primary cytosolic SOD of the lung, Manganese SOD [SOD2] which is concentrated in the mitochondria and extracellular SOD [SOD3] which is found bound to collagen and elastin surrounding

airways and blood vessels [30]. Human and animal studies indicate that the expression of the SOD family of enzymes can affect the development of both asthma and COPD. Oxidation and nitration of SOD2 is detected in the airways of asthmatics and correlated well with airway hyperresponsiveness [31]. Furthermore, SOD activity in the serum of asthmatics was lower than that found in normal serum. This decrease in SOD activity correlated well with oxidative markers, and, most importantly, with airflow limitation [32]. Recently, our laboratory has demonstrated that the transgenic expression of SOD1 in mice protects against the development of cigarette-smoke and elastase induced emphysema [33]. This effect appeared to be attributable to the ability of SOD1 to markedly reduce the influx of neutrophils in response to cigarette smoke exposure. Given these findings, the induction of enzymatic antioxidants is critical to protect the lung from the oxidative stress that occurs during asthma and COPD.

Within the epithelial lining fluid of the lung are numerous non enzymatic antioxidants that serve to protect the lungs from the damaging effects of cigarette smoke exposure. These include vitamins E and C, reduced glutathione, bilirubin, urate, albumin, surfactant protein D and mucin [34]. Dietary factors can affect the levels of these antioxidants and several epidemiologic studies have found positive correlations between the intake of antioxidants and lung function as determined by basic spirometry [35–38]. In addition, antioxidant consumption has been shown to decrease airway hyperresponsiveness in response to air pollutants and inhalational challenge in asthmatics [39, 40]. Animal studies demonstrate that these non enzymatic antioxidants have protective effects against the development of both asthma [41] and emphysema [42]. However, the use of exogenous antioxidants to treat these diseases is limited by the poor lung bioavailability of many of these agents [43]. Developing strategies to improve the delivery of antioxidants to target areas of the lung is critical. This is especially important given that antioxidant responses in these diseases are not consistent [44] and may not suffice to protect against the overwhelming exposure that occurs during cigarette smoke inhalation. Given the evidence of systemic oxidative stress in smokers [14, 45, 46], it is clear that the antioxidant response is inadequate to prevent the development of free radical injury. Strategies aimed at supplementing this response may have clinical benefits for patients.

Smoke-derived Oxidants and MAPK Induced Inflammation

Oxidants present in cigarette smoke can affect inflammation by regulating numerous redox sensitive signaling systems in the lung. The MAPK pathway is one important signaling cascade that can be affected by oxidants. MAPKs are highly conserved, eukaryotic signal transducing enzymes that respond to environmental stresses, as well as to plasma membrane receptor stimulation, by acting upon key molecular targets affecting gene transcription. MAP kinases, including extracellular signal-regulated kinases [ERKs], c-Jun N-terminal kinases [JNKs], and p38, are known to have important effects on immune responses in the lung. In asthma, CD4⁺ Th2 cells release cytokines [IL-4, -5, -9 and -13] that are involved in IgE production, airway eosinophilia and airway hyperresponsiveness [47]. Thus, they play a central role in the development of the disease. Several studies have shown that the MAPK signaling pathway exerts important effects on T cell activation. ERK1 signaling seems to be required for Th2 differentiation [48, 49]. In fact, Ras/ERK pathway activation in response to T cell antigen receptor stimulation may direct the differentiation of naïve T helper lymphocytes into the Th2 lineage. In addition to this important effect, ERK2 activation has been shown to facilitate the recruitment of eosinophils into the lung [50]. Similarly, JNK appears to have an important effect on the activation of inflammatory responses. JNK mediated phosphorylation of the transcription factor NFATc up regulates IL-4 production and induces Th2 differentiation [51, 52] and a specific JNK inhibitor was shown to reduce the influx of eosinophils and T lymphocytes into the lungs of ova challenged rats [53]. More recently, it

was demonstrated that the inhibition of JNK and ERK, but not p38, decreased lung inflammation in a murine asthma model [54]. Together, these results suggest that the modulation of T cell responses by MAPK signaling may be a critical factor in the pathogenesis of asthma.

Unlike asthma, neutrophils [55] and macrophages [56] are believed to be the key inflammatory cells in COPD. These cells can release powerful proteases that induce dysfunctional matrix remodeling in the lung. Several studies have shown that MAPK signaling can regulate the influx of these inflammatory cells in response to cigarette smoke exposure. In particular, p38 can augment lung inflammation by up regulating intracellular adhesion molecule-1 [ICAM-1] [57], TNF- α [58] and macrophage inflammatory protein-2 [MIP-2] [58] thus enhancing the chemotaxis of neutrophils and monocytes into the lung. In fact, p38 MAPK inhibition resulted in a decrease in the expression of proinflammatory cytokines and neutrophilia in an animal model of COPD [59]. Aside from these effects on inflammation, our laboratory has demonstrated that MAPK signaling can induce the expression of collagenase-1 in response to cigarette smoke exposure [60]. Thus, this pathway can regulate both the inflammatory and proteolytic responses that result in the structural changes that are seen in COPD.

While cigarette smoke can induce MAPK signaling in asthma and COPD, the disease it generates in these two subsets of patients is quite distinct. Though the exact mechanisms responsible for these differences have not been determined, it is likely that input from other signaling pathways in addition to host susceptibility factors may decide the type of inflammation that occurs. As an example, MAPK signaling can induce the synthesis and secretion of IL-4 [61]; however, this response may depend on genetic polymorphisms in the IL-4 gene that enhance the response to appropriate signaling [62]. In patients susceptible to COPD, on the other hand, polymorphisms in MAPK regulated genes such as IL-8 [63] may lead to a pronounced neutrophil inflammatory response. Future research is needed to better understand why MAPK signaling causes varied inflammatory responses in these distinct disease processes. A better understanding of these mechanisms may lead to more targeted strategies for treating these diseases.

Smoke-derived oxidants, through their effects on MAPK signaling, could have major effects on lung inflammation. Reactive oxygen species present in cigarette smoke such as peroxynitrite, H₂O₂ and O₂•⁻ were demonstrated to promote the phosphorylation and activation of ERK [60, 64], p38 [65] and JNK [66]. Several studies have examined the mechanism whereby reactive oxygen species activate MAPK pathways. This process is dependent on the activation of specific tyrosine kinases such as epidermal growth factor [EGF] receptor. Upon stimulation, the EGF receptor undergoes homodimerization or heterodimerization that leads to autophosphorylation and tyrosine kinase activation [67] [Figure 1]. This causes downstream activation of MEK-1 and Raf-1 and direct stimulation of MAPK proteins. Several studies have demonstrated that reactive oxygen species can initiate MAPK signaling by causing Src-dependent phosphorylation of the EGFR [68, 69]. In addition to their effects on cell surface receptors, oxidants can enhance MAPK signaling by inactivating protein tyrosine phosphatases [PTPs]. This family of proteins has a signature motif that contains a cysteine residue in its catalytic site. The oxidation of this cysteine residue inactivates PTPs and enhances tyrosine phosphorylation in response to environmental stimuli [70]. Thus, oxidants produced during cigarette smoke exposure can transiently inactivate the critical PTP[s] that provide an inhibitory constraint upon the system thereby amplifying the signaling response to exogenous stimuli.

The reversibility of intracellular signaling events is critical to ensure that the cell is able to adapt to changes in environmental stimuli. If reactive oxygen species irreversibly oxidize the

cysteine residue of PTPs, then the MAPK pathway within the cell would remain continually activated. However, crystallographic analysis shows that the oxidation of the cysteine residue induces profound changes in the architecture of PTPs [71, 72]. These changes protect PTPs from further oxidation while exposing it to intracellular reducing agents which can convert it back to its active, reduced form. The oxidation of MAPK phosphatases [MKPs], a member of the PTP family, leads to a decrease in MKP activity and sustained MAPK activation [73]. Furthermore, antioxidant treatment in mice increased MKP activity and attenuated the induction of MAPK signaling that occurred in response to Concavalin A treatment [73]. These results demonstrate the important role that oxidants play in regulating the MAPK pathway. Future work will need to identify which MKPs are oxidized and how this oxidation impacts on the activity of the entire family of MAPK proteins.

Smoke-derived Oxidants and the Activation of NF- κ B

NF- κ B is an important redox sensitive transcriptional regulator that consists of homo or heterodimers of the Rel family [usually p65 and p50]. Under basal conditions, NF- κ B is associated with its inhibitor I κ B in the cytosol. When the cell is stimulated, I κ B- α is phosphorylated by I κ B kinases [IKK] at two separate serine residues. This targets the molecule for ubiquitination and subsequent proteasomal degradation. Degradation of I κ B unmasks the nuclear localization sites of NF- κ B thus enabling it to translocate to the nucleus, bind promoter regions in the DNA and induce the transcription of over 150 target genes [74]. NF- κ B target genes primarily encode for proteins that participate in the host immune response. These include, for example, 27 different cytokines and chemokines, as well as immune recognition receptors [MHC molecules], proteins involved in antigen presentation and cellular adhesion molecules that are required for neutrophil adhesion and transmigration across blood vessel walls [75]. In vivo studies in mice confirm the importance of NF- κ B in the induction of inflammatory responses in the lung. Upon inhalational challenge, mice with deficient NF- κ B signaling have attenuation of inflammation, mucus cell metaplasia, inflammatory cytokines and serum IgE levels compared to matched controls [76]. Similarly, these mice have a marked decrease in lung neutrophilia and cytokine expression in response to LPS stimulation [77]. Together, these studies demonstrate that NF- κ B is a key modulator of inflammatory responses in the lung.

Several studies have demonstrated that redox factors can have profound effects on NF- κ B signaling. Treatment with lipid peroxidation products or depletion of reduced glutathione induces the ubiquitination and subsequent proteasomal degradation of I κ B [78, 79]. Similarly, H₂O₂ administration has been shown to produce the activation of NF- κ B in several cell lines [80–82]. Reactive oxygen species from cigarettes have the potential to induce NF- κ B through several distinct mechanisms [Figure 2]. As discussed above, oxidants can activate MAPK proteins which can then phosphorylate and activate I kappaB kinase [IKK]. Moreover, H₂O₂ can directly activate IKKs through phosphorylation of serine residues in their activation loops [83]. Both of these pathways will target I κ B for proteasomal degradation. Oxidants can also induce protein tyrosine kinases [PTKs] that phosphorylate I κ B and cause it to dissociate from NF- κ B without targeting it for proteasomal degradation [84]. In addition, recent data demonstrates that oxidants can mediate the direct phosphorylation of the p65 subunit of NF- κ B. This activates the complex and facilitates its binding to DNA [85]. The activation of NF- κ B is complex and the pathways involved are likely to differ amongst cell types which may explain the variable effects of oxidants on NF- κ B signaling that have been reported in the literature [86]. While these data demonstrate the important effects of oxidants on the induction of NF- κ B, much remains to be learned about the specifics of the oxidants and signaling pathways involved in this process.

The Role of Smoke-derived Oxidants on AP-1 Activation

AP-1 is a transcription factor that is composed of dimeric transcription elements containing fos, jun, and ATF subunits. c-Jun is the most potent transcriptional activator in this group while fos functions to stabilize jun and enhance its binding to promoter regions in DNA [87]. AP-1 activity is regulated by a host of factors including the transcription of fos and jun genes, the rate of turnover of these proteins, post-translation modification of fos and jun, and interactions with other transcriptional co-activators [88]. MAPK signaling is an important pathway that has been linked to AP-1 activation. ERK, p38 and JNK have all been shown to stimulate AP-1 signaling via phosphorylation of fos, jun or ATF subunits [89, 90]. In addition, ERK can induce fos expression by enhancing the binding of transcriptional co-activators to the promoter region of the gene [91]. Once activated, AP-1 can exert potent effects on the induction of proinflammatory genes particularly C-X-C chemokines [92]. AP-1 binding has been shown to enhance the expression of cytokines in alveolar macrophages [93] and lung epithelial cells [94]. This is important as these two cell types are responsible for the influx of inflammatory cells that occurs in response to cigarette smoke exposure [95]. Thus, control of AP-1 binding may have profound effects on smoke-induced inflammation in the lung.

The stimulation of MAPK signaling by oxidants can have potent effects on AP-1 activation. Treatment of macrophages with H₂O₂ was shown to enhance AP-1 signaling by triggering the phosphorylation of fos and jun while inducing the expression of fos [96]. This activation was attenuated by the use of specific ERK or JNK inhibitors demonstrating that the effects were mediated by the MAPK pathway. Similarly, oxidative stress turned on MAPK signaling which resulted in a sustained activation of AP-1 in mouse lung epithelial cells [97]. In addition to its effects on upstream activators like MAPK, oxidants can augment AP-1 signaling by directly up regulating the expression of AP-1 subunits. H₂O₂ treatment provoked a 50 fold increase in the levels of transcripts for c-fos and c-jun in epithelial cells [98]. The induction of AP-1 by smoke-derived oxidants can have important effects on the inflammatory responses of the lung. In a recent study, treatment of lung macrophages with cigarette smoke extract caused a marked increase in the release and expression of the proinflammatory chemokine CXCL8 from these cells [99]. This effect was oxidant related as co-treatment with the antioxidant, N-acetylcysteine, caused a concentration dependent decrease in the expression of CXCL8 from these cells. Cigarette smoke extract initiated the phosphorylation of c-jun which enhanced the binding of this subunit to the promoter region of the gene resulting in a potent increase in CXCL8 expression. Thus, these results provide direct links between cigarette smoke free radicals, AP-1 activation and post-exposure inflammatory responses.

The Regulation of Nrf2 by Cigarette Smoke Free Radicals

Nuclear factor erythroid derived 2, like 2 [nrf2] is a transcriptional element that regulates the response of cells to oxidative stress. This factor can activate transcription by binding to a specific DNA motif 5'-TGCTGAGTCAC-3' [100] that is found in the antioxidant responsive element [ARE]. This enhancer sequence regulates the expression of genes that protect the cell from oxidative injury. By binding to the ARE, Nrf proteins can induce the expression of antioxidant genes such as catalase, heme oxygenase, glutathione *S*-transferases [GST] [101] and NAD(P)H:quinone oxidoreductase [NQO1]. Thus, the regulation of Nrf activation can have critical effects on the antioxidant responses of the cell. It is theorized that oxidants can stimulate Nrf by activating kinases which phosphorylate the protein and cause it to dissociate from its cytosolic inhibitor, Keap-1. In addition, oxidants can oxidize the cysteine residue on Keap-1 which prevents binding to Nrf [102]. As a consequence of these effects, Nrf is free to translocate to the nucleus and induce the

expression of antioxidant genes. This oxidant-mediated pathway regulates Nrf activation and ensures that antioxidant genes are up regulated in a timely manner in response to oxidative stress.

The Effect of Nrf2 on Inflammatory Diseases of the Lung

The expression of Nrf2 has critical effects on lung inflammatory responses following cigarette smoke exposure. Mice deficient in Nrf2 had a marked increase in the number of macrophages detected in the lung lavage and tissue following cigarette smoke exposure [103]. The induction of antioxidants was significantly attenuated in the Nrf2^{-/-} mice which resulted in a striking increase in oxidative injury in these animals. Importantly, Nrf2^{-/-} mice exhibit increased susceptibility to cigarette smoke-induced emphysema. These results indicate that oxidants present in cigarette smoke can regulate the degree of inflammation and emphysema that occurs in response cigarette smoke exposure. Therefore, the ability of Nrf2 to modulate antioxidant expression can have a critical effect on the pathogenesis of this disease.

In addition to the effects on smoke-induced responses in the lung, Nrf2 can decrease oxidative injury and down regulate the inflammatory responses that occur in asthma. Nrf2^{-/-} mice exhibit increased lung infiltration with macrophages, lymphocytes, eosinophils and neutrophils after ova albumin inhalational challenge [104]. Moreover, these mice had elevated mucus cell hyperplasia and an increase in airway reactivity in response to acetylcholine challenge [104]. Again, these responses were associated with a deficient antioxidant response and an increase in the level of oxidative damage measured in the lung of these animals. This study demonstrates that abnormal Nrf signaling can lead to a defect in antioxidant responses that can exacerbate the key pathogenic features [i.e. inflammation, airway reactivity and mucus hyperplasia] of this disease.

Effects of Cigarette Smoke Oxidants on Chromatin Remodeling

Chromatin remodeling is a dynamic process in the cell that regulates gene silencing and expression. The primary subunit of chromatin, the nucleosome, is composed of an octamer of four core histones, two H2A–H2B dimers, and an H3–H4 tetramer surrounded by 146 bp of DNA [105]. Acetylation of lysine residues on the histone proteins by histone acetyltransferase [HAT] can cause unwinding of the DNA and allow transcription to occur [106]. Conversely, histone deacetylases [HDACs] remove these acetyl groups thus resulting in recoiling of the DNA. Alterations in the balance of acetylation/deacetylation can have significant effects on the transcription of genes involved in the immune response of the lung. Nuclear receptor co-activators like steroid receptor co-activator-1 [SRC-1], ATF-2 and CREB binding protein [CBP]/p300 possess intrinsic HAT activity. These co-activators can facilitate the binding of RNA polymerase II which is believed to play an important role in the induction of numerous proinflammatory genes [107]. An increase in HAT activity has been detected in macrophages [108] and lung tissue [109] isolated from asthmatics and is associated with the expression of proinflammatory cytokines [108]. These studies demonstrate that HAT activity may contribute to the inflammatory process that occurs in the airways of asthmatics.

The Impact of HDACs on Lung Inflammation

The HDAC family of enzymes plays an important role in the immune response of the lung. These enzymes hypoacetylate DNA and thus prevent the binding of transcriptional activators such as NF- κ B and AP-1. Thus, they can prevent transcriptional activators from inducing the expression of genes linked to inflammation. A recent study demonstrated that treatment of lung epithelial cells with dexamethasone prevented the histone acetylation that

occurred following IL-1 β treatment [110]. This decrease in histone acetylation was due to the ability of dexamethasone to recruit HDAC2 to the p65-CBP HAT complex and to directly inhibit CBP associated HAT activity. HAT inhibition resulted in a marked decrease in the IL-1 β mediated expression of proinflammatory cytokines. In fact the authors demonstrated that 50% of the glucocorticoid action in repressing IL-1-stimulated cytokine release was mediated by HDACs [110]. This data suggests an important role for HDACs in the anti-inflammatory properties of glucocorticoids.

In addition to their effects on the acetylation of nuclear DNA, HDACs can modulate inflammation by affecting the duration of NF- κ B activity in the nucleus. HDACs mediate this effect through their action on the RelA subunit of NF- κ B. Upon stimulation, the RelA subunit together with the p50 subunit translocate to the nucleus and bind to the promoter regions of proinflammatory genes. Once in the nucleus, the RelA subunit is acetylated by CBP and p300 co-activators. The effect of this acetylation is to protect RelA from binding to its inhibitor I κ B α . HDAC3 by deacetylating RelA allows the subunit to bind to I κ B α thus targeting the NF- κ B complex for export from the nucleus [111]. Thus, the HDAC mediated deacetylation of RelA has the effect of limiting the inflammatory effects of exogenous stimuli on the cell. Given the known effects of NF- κ B on inflammation, this mechanism may function to limit the smoke-induced immune responses in the lung.

Recent data suggests that HDACs have an important role in the pathogenesis of COPD and asthma. A marked reduction in the activity of HDACs was noted in lung tissue samples resected from COPD patients [109]. This decreased activity correlated with an increase in the histone acetylation of the IL-8 promoter, increased expression of the IL-8 cytokine and overall disease severity [109]. As noted previously, corticosteroids inhibit HAT activity and recruit HDAC2 to the inflammatory gene complex thereby decreasing the expression of pro-inflammatory genes [110]. In COPD, the inactivation of HDACs counteracts these effects leading to corticosteroid resistance [112]. While reductions in HDAC activity have been noted in asthma, they tend to be much less than those observed in COPD [108] [113]. However, asthmatics who smoke have a much greater reduction in HDAC activity [114] and this could explain the disease severity and steroid resistance that is observed in this group of patients [115]. As discussed below, it is probable that the increased and persistent smoke-related oxidative burden can inactivate HDACs and thereby generate inflammation that is refractory to standard therapy. These results suggest that HDACs can have a determinant role on the inflammatory responses that are central to the development of these diseases. Indeed, deficiencies in intrinsic HDAC activity may confer upon an individual greater susceptibility to the onset of these inflammatory disorders.

Effect of Oxidants on HDAC Levels and Activity

Given the importance of the HAT:HDAC balance on the regulation of inflammatory responses of the lung, factors that affect this balance can have a significant effect on the pathogenesis of asthma and COPD. There is significant evidence to suggest that cigarette smoke-derived oxidants can alter the HAT:HDAC balance thereby up regulating the immune reaction to cigarette smoke exposure. Treatment of lung epithelial cells with H₂O₂ has been shown to enhance oxidative stress as judged by the depletion of intracellular levels of reduced glutathione [116]. This oxidative stress was associated with an increase in HAT activity, enhanced nuclear NF- κ B and AP-1 binding, and an up regulation of proinflammatory cytokine expression [116]. Similar effects were noted on studies of human bronchial epithelial cells [117]. Further studies revealed that H₂O₂ can also augment histone acetylation and NF- κ B activation by decreasing the levels and activity of HDAC in these cells [118]. This decrease in HDAC activity has been associated with the nitration of tyrosine residues which are present in the HDAC molecule [119] which is significant since

oxidant-mediated tyrosine nitration has been noted to be significantly increased in both COPD and asthmatic patients [120]. Taken together, these data provide evidence that oxidants present in cigarette smoke may modulate immune responses by affecting HAT/HDAC signaling in the lung.

Despite the importance of chromatin remodeling on the activation of proinflammatory genes, few studies have directly examined the effect of cigarette smoke on this crucial process. A recent study in lung epithelial cells demonstrated that cigarette smoke condensate increased HAT activity and decreased HDAC activity thus resulting in the increased acetylation of histone [H4] proteins in the nucleus [118]. Smoke exposure not only decreased the levels of HDAC present but also led to oxidative modifications that markedly decreased the activity levels of the protein. Interestingly, smoke exposure did not induce NF- κ B and cytokine expression in these epithelial cells. This is in contrast to data from T cells [121] and macrophages [122] which demonstrates both NF- κ B activation and cytokine up regulation following cigarette smoke exposure. These results suggest that the ultimate effects of changes in the balance of HAT:HDAC activation depends on critical co-factors that can vary from cell to cell. Cigarette smoke exposure, however, did lead to a direct increase in histone acetylation in rat lungs [123]. This increase was associated with a decrease in HDAC activity and an enhancement in NF- κ B mediated signaling in these animals. Coupled with existing correlative data from humans [109], these results suggest that cigarette smoke amplifies lung inflammation by increasing HAT while decreasing HDAC activity in the lung.

Conclusions

The lung, unlike any other organ, is directly exposed to oxygen in the ambient air. Thus, it experiences enhanced oxidant stress compared to other organs. Cigarette smoke contributes additional oxidants and stimulates inflammation further augmenting free radical production. As we have discussed, this enhanced oxidant burden can trigger lung inflammation in a multifactorial process. The burning of a cigarette releases an enormous quantity of free radicals that are then directly inhaled into the lung. These free radicals can overwhelm the local antioxidant defenses and induce cell signaling events that ultimately promote an inflammatory response. The oxidant/antioxidant imbalance can lead to the stimulation of cell surface receptors and inactivation of phosphatases resulting in the amplification of MAPK, NF- κ B and AP-1 signaling pathways thereby augmenting inflammation. In addition, the oxidant-mediated activation of HATs and inactivation of HDACs renders nuclear DNA more accessible to transcriptional activation. These transcriptional factors can then up regulate the expression of proinflammatory cytokines and cellular adhesion molecules that promote the infiltration of inflammatory cells into the lung. Once these cells are present, they release proteases, oxidants and vasoactive mediators that destroy the lung architecture, enhance mucus release and induce bronchoconstriction. Thus, oxidants present in cigarette smoke can initiate the pathologic and physiologic features that categorize both asthma and COPD.

Given the importance of oxidants in the pathogenesis of these inflammatory lung disorders, investigators have attempted to use antioxidants to treat patients with asthma and COPD. Several recent clinical trials for both asthma [124, 125] and COPD [43] have failed to demonstrate any benefit of antioxidant treatment. These negative studies have led some researchers to question the role of oxidants in smoke-related lung diseases. A major shortcoming of these studies, however, is that they failed to measure the effect of supplementation on the antioxidant status of the lung. For example, after N-acetylcysteine is ingested it first must be converted into glutathione and then must be secreted into the epithelial lining fluid in order to protect the lung from smoke-derived oxidants. The amount

of orally administered N-acetylcysteine that gets converted into glutathione within the lining fluid of the lung is extremely small. In fact, the dosage used in the described study, 600 mg/day, was shown to be ineffective in increasing NAC, cysteine, or glutathione levels in the bronchoalveolar lavage fluid of healthy volunteers [126].

Moreover, even if overall antioxidant levels are elevated in the lung, they may not be sufficient to prevent the onset of oxidative damage. This is a concept that is well established in the protease field. Campbell et al demonstrated that even in a system with an excess of protease inhibitors, significant proteolysis can occur in the direct periphery of inflammatory cells [127]. In fact, small changes in anti-protease concentration resulted in a disproportionate increase in the range of proteolysis. The concentration of proteases at the site of release is supraphysiologic and thus overwhelms the local anti-protease defenses. The same is likely to be true for smoke-related oxidative injury. With each puff of a cigarette, the lung is exposed to a tremendous oxidant burden that may supersede local antioxidant levels. If this oxidant imbalance occurs even transiently, it may be sufficient to trigger the above mentioned cell signaling pathways that result in the influx of inflammatory cells.

In conclusion, smoke-derived oxidants can overcome lung antioxidants, activate cell signaling and trigger the influx of inflammatory cells into the lung. This oxidant-mediated inflammatory cell infiltration is a key determinant in the development of pulmonary disorders such as asthma and COPD. While, antioxidant trials have failed to convincingly demonstrate a therapeutic effect, they have been limited by their inability to effectively augment the lung antioxidant defenses. Future research is needed both to better define the effects of smoke-derived oxidants on lung inflammation and to determine the most efficacious strategies for generating significant antioxidant protection in the lung. The results of this research may lay the foundation for the effective use of antioxidants as a tool to prevent smoke-related lung inflammation.

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Abbreviations

COPD	chronic obstructive pulmonary disease
MAPK	mitogen activated protein kinase
PTP	protein tyrosine phosphatase
MKP	MAPK phosphatase
HAT	histone acetyltransferase
HDAC	histone deacetyltransferase
IL	interleukin
SOD	superoxide dismutase
NFAT	nuclear factor of activated T cells
JNK	c-Jun NH ₂ -terminal kinase
ERK	extracellular regulated kinase
NF-κB	nuclear factor kappa B
MIP	macrophage inflammatory protein

TNF	tumor necrosis factor
ICAM	intracellular adhesion molecule
EGFR	epidermal growth factor receptor
GST	glutathione <i>S</i> -transferase
ATF	activating transcription factor
SRC	steroid receptor co-activator
Nrf	Nuclear factor erythroid derived
NAC	n-acetylcysteine
CREB	cyclic AMP response element binding protein
CBP	CREB binding protein
MEKK	mitogen-activated protein kinase kinase kinase

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Figure 1. The Effect of Smoke-derived Oxidants on MAPK Signaling

Oxidants present in cigarette smoke can induce the dimerization of the EGF receptor [arrow]. This stimulates the phosphorylation and activation of Ras, Raf and MEKK. MEKK phosphorylates and activates MAPK proteins. Oxidants can maintain MAPK proteins in their activated state by inactivating MKPs [blocked arrow]. The stimulate MAPK proteins can activate c-Jun and promote AP-1 signaling as well as acting on numerous other transcriptional factors. This leads to the induction of genes that promote the influx of inflammatory cells in the lung.

Figure 2. The Effect of Smoke-derived Oxidants on NF-κB Signaling

Reactive oxygen species in cigarette smoke activate IKK while inactivating IκB Phosphatase. This has the effect of phosphorylating IκB and targeting it for proteasomal degradation. In addition, smoke-derived oxidants stimulate PTK which then phosphorylates p50 and enhances its binding to DNA. NF-κB signaling increases the expression of numerous proinflammatory genes.