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Biochemical Pharmacology 68 (2004) 1255–1267
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Redox modulation of chromatin remodeling: impact on histone acetylation and deacetylation, NF-κB and pro-inflammatory gene expression

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Received 23 February 2004; accepted 17 May 2004

Abstract

Reactive oxygen species (ROS), either directly or via the formation of lipid peroxidation products, such as 4-hydroxy-2-nonenal, acrolein and F_2-isoprostanes, may play a role in enhancing inflammation through the activation and phosphorylation of stress kinases (JNK, ERK, p38) and redox-sensitive transcription factors such as NF-κB and AP-1. This increases the expression of genes regulating a battery of distinct pro-inflammatory mediators. Acetylation by histone acetyltransferase (HAT) of specific lysine residues on the N-terminal tail of core histones, results in uncoiling of the DNA and increased accessibility to transcription factor binding. In contrast, histone deacetylation by histone deacetylase (HDAC) represses gene transcription by promoting DNA winding thereby limiting access to transcription factors. Oxidative stress activates NF-κB resulting in expression of pro-inflammatory mediators through the activation of intrinsic HAT activity on co-activator molecules. In addition, oxidative stress also inhibits HDAC activity and in doing so enhances inflammatory gene expression which leads to a chronic inflammatory response. Oxidative stress can also increase complex formation between the co-activator CBP/p300 and the p65 subunit of NF-κB suggesting a further role of oxidative stress in chromatin remodeling. The antioxidant and/or anti-inflammatory effects of thiol molecules (glutathione, *N*-acetyl-L-cysteine and *N*-acystelyn), dietary polyphenols (curcumin-diferuloylmethane and resveratrol), the bronchodilator theophylline and glucocorticoids have all been shown to play a role in either controlling NF-κB activation or chromatin remodeling through modulation of HDAC activity and subsequently inflammatory gene expression in lung epithelial cells. Thus, oxidative stress regulates both signal transduction and chromatin remodeling which in turn impacts on pro-inflammatory responses in the lungs.
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Keywords: Reactive oxygen species; Glutathione; Lipid peroxides; NF-κB; Corticosteroids; Histone acetylation; Histone deacetylase; Lungs

Abbreviations: A549, alveolar epithelial cells; AP-1, activator protein-1; ATF-2, activating factor-2; BALF, bronchoalveolar lavage fluid; COPD, chronic obstructive pulmonary disease; CBP, cyclic AMP response element binding (CREB)-binding protein; COX-2, cyclooxygenase; EMSA, electrophoretic mobility shift assay; ERK, extracellular signal regulated kinase; GSH, glutathione; GSSG, glutathione disulfide; 4-HNE, 4-hydroxy-2-nonenal; H_2O_2, hydrogen peroxide; H4, histone 4; ICAM-1, intracellular adhesion molecule-1; IL-1, interleukin-1; IL-8, interleukin-8; iNOS, inducible nitric oxide synthase; HDAC, histone deacetylase; HAT, histone acetyltransferase; IκB, inhibitory κB; IKK, I-kappaB kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen activated protein kinase; NAC, *N*-acetyl-L-cysteine; NAL, *N*-acystelyn; NF-κB, nuclear factor-kappaB; $O_2^{\bullet -}$, superoxide anion; $^{\bullet}OH$, hydroxyl radical; p/CAF, CBP/p300 associated factor; ROS, reactive oxygen species; TSA, trichostatin A; TNF-α, tumor necrosis factor-α

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doi:10.1016/j.bcp.2004.05.042

1. Introduction

Biological systems are continuously exposed to oxidants, either generated endogenously by metabolic reactions (e.g. from mitochondrial electron transport during respiration or during activation of phagocytes) or exogenously, such as air pollutants or cigarette smoke. Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet -}$) and the hydroxyl radical ($^{\bullet}OH$) are highly unstable species with unpaired electrons, capable of initiating oxidation. ROS causes oxidation of proteins, DNA, and lipids which can cause direct lung injury or induce a variety of cellular responses, through the generation of secondary metabolic

reactive species. ROS can alter remodeling of extracellular matrix and blood vessels, stimulate mucus secretion, cause apoptosis, and regulate cell proliferation [1,2]. Alveolar repair responses and immune modulation in the lung are also influenced by ROS [1,2]. Over the past decade, ROS has been implicated in initiating inflammatory responses in the lungs through the activation of transcription factors such as nuclear factor-kappaB (NF-κB) and activator protein-1 (AP-1) causing chromatin remodeling and gene expression of pro-inflammatory mediators [3,4]. These transcription factors are regulated by the activation of Iκ-B kinase, mitogen activated protein (MAP) kinase pathways and phosphoinositide 3-kinase (PI-3-kinase, PI-3K) pathways. Recently, it has been shown that oxidative stress and redox status of the cells can also regulate nuclear chromatin remodeling (histone acetylation/deacetylation) leading to gene expression of pro-inflammatory mediators [4,5].

This commentary discusses the role of oxidative stress and redox status of the cells in cell signaling, NF-κB activation and its involvement in chromatin remodeling, and pro-inflammatory gene transcription in inflammation.

1.1. ROS-mediated lipid peroxidation products and their role in biochemical processes

ROS can originate from both environmental sources (e.g. ozone and cigarette smoke) and inflammatory cells. Cell-derived ROS, such as $O_2^{\bullet -}$ and $^{\bullet}OH$ are generated and released by activated inflammatory cells. They are produced intracellularly by several mechanisms such as, mitochondrial respiration, the NADPH oxidase system, xanthine/xanthine oxidase, but primarily through NADPH oxidase. ROS are highly reactive and when generated close to cell membranes, oxidize membrane phospholipids (lipid peroxidation) which can lead to the generation and accumulation of lipid peroxidation products, such as malondialdehyde, 4-hydroxy-2-nonenal (4-HNE), acrolein and F_2-isoprostanes (Fig. 1). The peroxidative breakdown of polyunsaturated fatty acids impairs membrane function, inactivates membrane-bound receptors and enzymes and increases tissue permeability which have been implicated in the pathogenesis of many forms of lung injury [6]. There is increasing evidence that aldehydes, generated endogenously during the process of lipid peroxidation, are involved in many of the pathophysiological events associated with oxidative stress in cells and tissues [7]. In addition to their cytotoxic properties, lipid peroxides are increasingly recognized as being important in signal transduction for a number of important events in the inflammatory response [8].

1.1.1. F_2-isoprostanes

Isoprostanes (members of the F_2-isoprostanes) are just one of the non-enzymatic (non-cyclooxygenase dependent) lipid peroxidation products of arachidonic acid

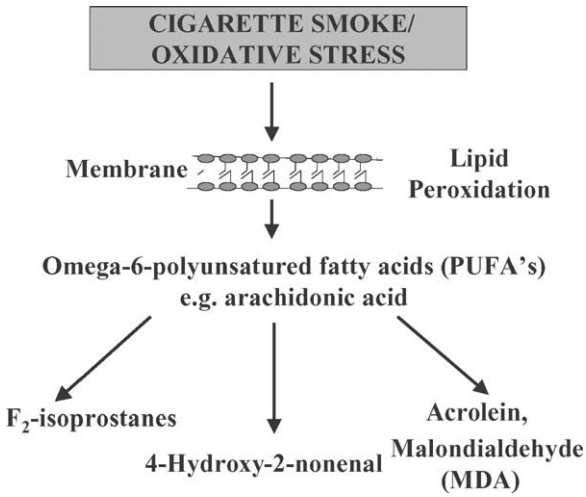

Fig. 1. Membrane lipid peroxidation of polyunsaturated fatty acids leading to generation of various aldehydes.

[9,10]. F_2-IsoPs are initially formed in situ by oxidation of phospholipids and then released by the action of phospholipases. F_2-isoprostane is a potent smooth muscle cell constrictor and a mitogen. It modulates platelet activity as well as other cell functions in vitro via membrane receptors (thromboxane A_2) for prostaglandins [9,10].

1.1.2. 4-Hydroxy-2-nonenal

4-Hydroxy-2-nonenal is a highly reactive and specific diffusible end-product of lipid peroxidation. It also acts as a chemoattractant for neutrophils in vitro and in vivo [11]. 4-HNE-modified protein levels are increased in airway and alveolar epithelial cells, endothelial cells and neutrophils in subjects with airway obstruction compared to subjects without airway obstruction [12]. 4-HNE is known to induce/regulate various cellular events such as proliferation and growth inhibition [13], T cell apoptosis [14] and activation of signaling pathways [8,15]. 4-HNE has a high affinity towards cysteine, histidine and lysine residues forming direct protein-adducts and thereby altering protein function. Moreover, 4-HNE has also been reported to activate glutathione (GSH) synthesis via induction of the γ-glutamyl cysteine ligase (GCL) gene (a key enzyme for GSH synthesis) and a variety of pro-inflammatory genes, such as IL-8, MCP-1, COX-2, EGFR, MUC5AC, etc., suggesting that 4-HNE works as a signaling molecule in gene transcription [2,8,11,13–15].

1.1.3. Acrolein

Acrolein is another thiol reactive, αβ-unsaturated aldehyde which is present in various environmental sources. The largest source of acrolein is cigarette smoke [16]. Inhalation of acrolein is known to induce changes in rat lung structure and function. Previous studies have shown that acrolein exposure is known to deplete glutathione and inhibit the activity of various glutathione redox system enzymes in the nasal mucosa of rats [17,18] and in alveolar

A549 epithelial cells in vitro [19]. Acrolein exposure of 2 ppm to rats causes bronchioles to be filled with desquamized cells along with isolated peribronchial monocytes [20]. Indeed, a more recent study has shown that acrolein can lead to post-translational modification of extracellular matrix proteins causing macrophage adhesion to these modified proteins and their subsequent activation [21]. Exposure to acrolein has also been shown to reduce ciliary beat frequency in cultured bovine bronchial epithelial cells. In vitro exposure of bovine tracheal epithelial cells to acrolein caused increased release of a series of eicosonoids, such as PGE_2, $PGF_2\alpha$, etc. [18,19].

1.2. Oxidative stress and redox regulation of NF-κB

The NF-κB/Rel family complex is a redox-sensitive transcription factor composed of several key regulatory molecules controlling expression of many inflammatory and protective/stress response genes. NF-κB exists as a heterodimeric complex usually of p50 and p65/RelA subunits. In unstimulated cells NF-κB is found in the cytoplasm as an inactive non-DNA-binding form, associated with an inhibitor protein called inhibitory κB (IκB) which masks the nuclear translocation signal and so prevents NF-κB from entering the nucleus. Upon cell stimulation with various NF-κB inducers, IκB-α is rapidly phosphorylated on two serine residues, which targets the inhibitor protein for ubiquination by the E3 ubiquitin-ligases (E3RSIκB) and subsequent degradation by the 26S proteasome. The released NF-κB dimer can then be translocated into the nucleus and activate target genes by binding with high affinity to κB elements in their promoters [4,22]. A model was proposed, primarily based on studies with antioxidants, that introduced the idea that NF-κB was redox-sensitive and that its activation was due to the production of ROS by oxidants [4,22]. Oxidative stress including exposure to lipid peroxidation products or depletion of reduced glutathione causes rapid ubiquitination and phosphorylation with subsequent degradation of the IκB complex, a critical step for NF-κB activation [23,24]. Under reducing conditions, such as an increase in intracellular GSH following treatment with *N*-acetyl-L-cysteine (NAC), the phosphorylation of a serine group (ser 32) on IκB-α following TNF-α treatment is inhibited, leading to the downregulation of NF-κB in endothelial cells [25]. Therefore, it is possible that oxidative stress and/or an imbalance in GSH redox status may directly stimulate the activity of IκB-α kinase. Alternatively, elevated GSH levels may inhibit IκB-α kinase activity. It is also likely that changes in intracellular GSH redox status during oxidative stress may affect the proteosome enzymatic activity that leads to the activation of NF-κB [26]. Overall, oxidative stress favors the activation and translocation of NF-κB to the nucleus, and nuclear GSH (reducing environment) facilitates the binding of NF-κB to DNA. The mechanism of activation of NF-κB under oxidative stress and in altered redox GSH status may be cell-specific and distinct from physiological activators such as TNF-α and IL-1β, since diamide, which oxidizes GSH to GSSG and H_2O_2 is unable to activate NF-κB in certain cell types [27]. Several novel mechanisms have been proposed for H_2O_2-induced activation of NF-κB, which include tyrosine phosphorylation of IκB [28] and, more recently, activation of I-kappaB kinase (IKK) by H_2O_2 [29]. New data also reported IκB-independent mechanisms of activation of NF-κB where phosphorylation of p65 NF-κB by various kinases had an effect on the transactivation activity of NF-κB, independently of nuclear translocation. Although not explored yet, these new pathways may be critical to the H_2O_2-induced activation of NF-κB.

1.3. Involvement of oxidative stress and lipid peroxidation products in cell signaling and gene transcription

ROS can lead to the activation of various cell signaling pathway components. Examples include the extracellular signal regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38 kinase, PI-3K/Akt, via sensitive cysteine rich domains and the sphingomyelinase-ceramide pathway, all of which lead to increased gene transcription [4,5,30,31]. Indeed, activation of members of the MAPK family triggers the transactivation of transcription factors, such as c-Jun, activating transcription factor-2 (ATF-2), cyclic AMP response element binding proteins (CREB)-binding protein (CBP) and Elk-1 [30–32]. This eventually results in chromatin remodeling and the expression of a battery of distinct genes that can regulate pro-inflammatory, pro-apoptotic and antiproliferative responses. Likewise, lipid peroxidation products have also been shown to act as a signal for activation of transcription factors and gene expression, leading to both an inflammatory [8,15] as well as a protective/stress response. The latter has been exemplified by a couple of studies. The first study showed that the induction of γ-glutamylcysteine ligase or γ-glutamylcysteine synthetase (γ-GCS) may be an important adaptive response of the alveolar epithelium when under attack by oxidative stress and lipid peroxidation products such as 4-HNE [33]. The second more recent study by Sano et al. have shown that another antioxidant, the thioredoxin gene, is induced by 4-HNE in response to LPS challenge in mice thereby providing endotoxin tolerance [34].

1.4. Role of ROS in chromatin remodeling and pro-inflammatory gene transcription: epigenetics

Epigenetics is defined as the inheritance of information on the basis of gene expression in contrast to 'genetics', which is described as the inheritance of information on the basis of DNA sequence. Histone acetylation/deacetylation and DNA methylation are two important epigenetic events that play an important role in inflammatory lung responses.

1.4.1. Histone acetylation and deacetylation in chromatin remodeling

Many factors, including specific DNA sequences, histones, non-histone chromosomal proteins, transcriptional activators/repressors and the transcription machinery are all necessary for the establishment of an active transcription complex [35]. Condensation of eukaryotic DNA in chromatin suppresses gene activity through the coiling of DNA on the surface of the nucleosome core and the folding of nucleosome assemblies, thus decreasing the accessibility to the transcriptional apparatus [36]. Tightly bound DNA around a nucleosome core (comprising the histone proteins H2A, H2B, H3 and H4), suppresses gene transcription by decreasing the accessibility of transcription factors, such as NF-κB and AP-1 to the transcriptional complex. Acetylation of lysine residues on the N-terminal tails of the core histone proteins results in uncoiling of the DNA, allowing increased accessibility for transcription factor binding [37]. Acetylation of lysine (K) residues on histone 4 (K5, K8, K12, K16) is thought to be directly related to the regulation of gene transcription [37,38]. However, selective recognition of these acetylated lysines within the histone by other proteins containing bromodomains may add a further level of transcriptional regulation [39]. Histone acetylation is reversible and is regulated by a group of acetyltransferases (HATs) which promote acetylation, and deacetylases (HDACs) which promote deacetylation.

The nuclear receptor coactivators, steroid receptor coactivator 1 (SRC-1), CREB-binding protein (CBP)/adenoviral protein E1A (p300) protein, CBP/p300 associated factor (P/CAF), and ATF-2, all possess intrinsic HAT activity (Fig. 2) [40–42]. Of these, CBP/p300 and ATF-2, which are regulated by the p38 MAP kinase pathway, are vital for the co-activation of several transcription factors, including NF-κB and AP-1 in the transcription machinery [40–43]. These activation complexes act with RNA polymerase II to initiate transcription (Fig. 3) [44–46]. Thus, it

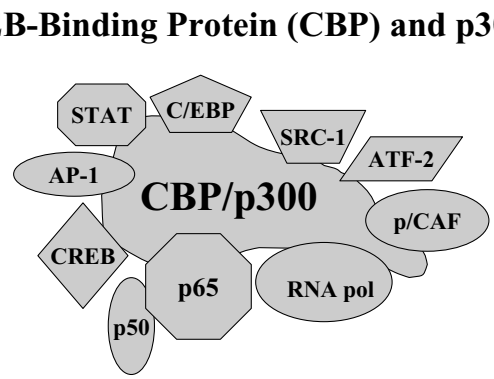

Fig. 2. CBP/p300 co-activator transcription initiation complex. CBP recruits transcriptional co-activators and acts as an integrator of signaling pathways, links transcription factors and co-activators to the basal transcriptional machinery and stabilizes the transcriptional preinitiation complex.

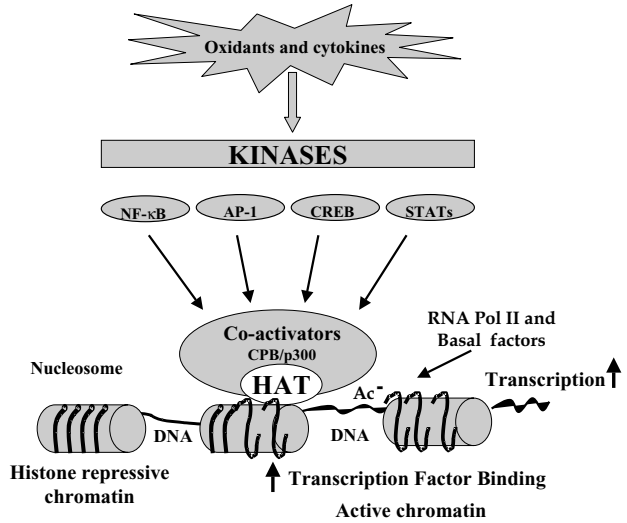

Fig. 3. Histone acetylation and deacetylation: oxidative stress and other stimuli, such as cytokines, activate various signal transduction pathways (JAK, JNK and IKK) leading to activation of transcription factors, such as NF-κB, AP-1, CREB and STAT proteins. Binding of these transcription factors leads to recruitment of CBP and/or other co-activators to the transcriptional initiation complex on promoter region of various genes. Activation of CBP leads to acetylation (Ac) of specific core histone lysine residues by an intrinsic acetyltransferase activity (HAT). Histone acetylation (active chromatin) leads to loosening of the nucleosome which enables access to basal factors and RNA polymerase II for gene transcription to occur.

is likely that histone acetylation of H4 via CBP/p300 and/or ATF-2 has a significant role in the activation of NF-κB/AP-1-mediated gene expression for pro-inflammatory mediators [41,44,45], although the precise molecular mechanisms are still not fully understood.

The family of HDAC enzymes consists of 17 isoforms grouped into three families [47]. Class I HDACs (HDAC1–3 and 8) reside almost exclusively in the nucleus, whereas class II HDACs (HDAC4–7, 9–11) are able to shuttle between the nucleus and cytoplasm in response to certain cellular signals. The third HDAC family consists of sirtuins 1–6 and their function is not yet fully understood. A common feature of HDACs is the ability to remove acetyl moieties from the ϵ-acetamido group on lysine residues within histones, resulting in condensation of DNA thereby silencing gene transcription. Various members of the classes I and II HDAC families have been shown to play a role in the regulation of cell proliferation and differentiation [47,48]. More recently, however, HDAC2 has been reported to function in corticosteroid-mediated anti-inflammatory mechanisms [49]. Furthermore, there is increasing evidence to suggest that HDACs play an important role in regulating pro-inflammatory responses. Many of these HDACs are differentially expressed and regulated in different cell types. It is highly likely therefore, that this differential expression and interaction between the various HDAC isoforms could fine tune the repression of gene expression these HDACs are able to exert. HDACs not only cause the inhibition of gene transcription, but also directly

affect the nuclear activity of transcription factors such as NF-κB. The duration of the NF-κB nuclear activation has been shown to be dependent upon the activity of HDAC3 [50], which provides an acetylation balance dependent mechanism for the regulation of NF-κB-mediated transcription. Once acetylated active NF-κB dimers are present in the nucleus, they will scan the chromatin for newly exposed binding sites. This is confirmed by Saccani et al., who have shown dual waves (two temporally distinct phases) of recruitment of NF-κB to target promoters by LPS stimulation in the Raw 264.7 murine macrophage cell line [51]. They suggested that a subset of target genes, whose promoter is already heavily acetylated (H4 acetylation) before stimulation, is constitutively and immediately accessible to NF-κB and is transcribed immediately (MnSOD, MIP2, IκB-α, IL-2) after NF-κB recruitment, whereas other target genes are not immediately accessible to NF-κB (MCP-1, RANTES, IL-6). Recruitment of NF-κB (p38-dependent) to late accessible gene promoters occurs after nuclear entry and is preceded by the formation of an initial transcription factor complex that directs the hyperacetylation of the promoter and makes it accessible to NF-κB [52]. This shows the selectivity of stimulus-specific p38-dependent and NF-κB-mediated histone acetylation leading to a subset of gene transcription (Fig. 4). This temporal control of gene expression by HDAC may also be regulated at another level. Viatour et al. have shown that HDAC1 and 3 can complex with free cytoplasmic Iκ-Bα sequestering this complex to the cytoplasm and stimulating NF-κB-independent transcription [53]. Moreover, this could have the additional impact of removing HDAC3 from the nucleus thereby maintaining the acetylated state of NF-κB.

1.4.2. DNA/histone methylation in chromatin remodeling

During the last few years it has become clear that DNA methylation is an essential component of epigenetic phenomena, such as genomic imprinting. Evidence suggests that two modifications (histone acetylation and DNA/histone methylation) are dynamically and physically linked and are involved in regulating imprinted genes—a subset of genes whose expression depends on their parental origin. Histone/DNA methylation is a very effective mechanism to turn a gene off and alter gene expression within a cell. Thus, aberrant methylation of cytosine residues in the cytosine/guanosine (CpG) dinucleotide patterns plays a critical role in certain human diseases [54]. Certain genes may be inappropriately switched off in inflammatory diseases, due to inappropriate methylation.

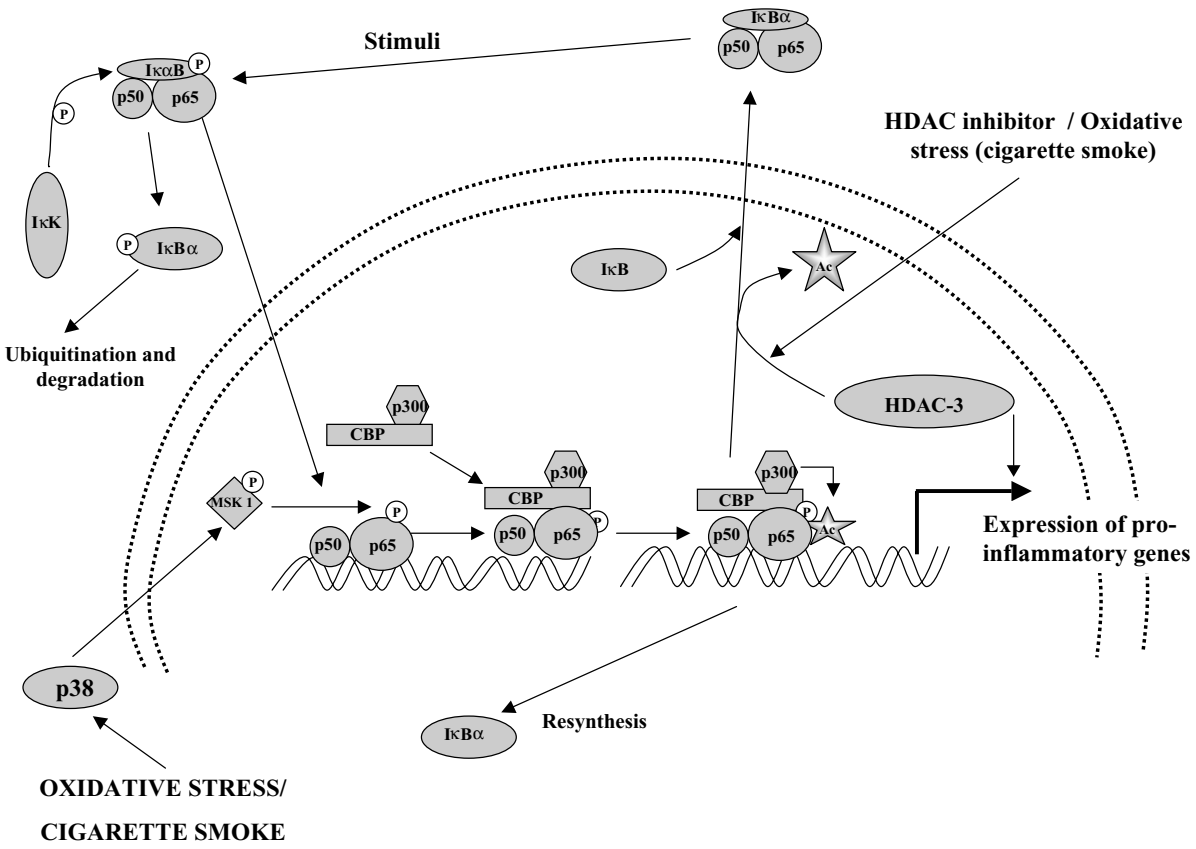

Fig. 4. Proposed mechanisms of oxidant-mediated phosphorylation and/or acetylation of p65 NF-κB leading to expression of pro-inflammatory genes. Oxidative stress may activate the p38 MAP kinase signaling pathway leading to recruitment of CBP in the transcription initiation complex. HDAC inhibition will also lead to increased acetylation of p65 subunit.

This abnormal DNA methylation of specific gene(s) may be disease-specific.

The methyl-cytosine binding protein, MeCP2, recruits HDAC to methylated DNA as well as to methylated histone, resulting in histone deacetylation, chromatin condensation and transcription silencing [54,55]. Recently, Kagoshima et al. have demonstrated that inhibition of HDACs and DNA methylase leads to upregulation of pro-inflammatory mediator GM-CSF by IL-1β in lung epithelial cells [56]. This epigenetic phenomenon is also demonstrated by showing an aberrant promoter methylation of multiple genes, including GSTP1, p16 (INK4a) tumor suppressor gene and *O*(6)-methylguanine-DNA methyltransferase DNA repair gene and death-associated protein (DAP) kinase, in sputum and bronchial epithelium and brush biopsy samples of current/ex-smokers and subjects at high risk for developing lung cancer [57,58]. Similarly, Soria et al. have demonstrated promoter hypermethylation of various genes, including p16, death-associated protein kinase and GSTP1, in bronchial brush samples obtained from ex-smokers [59]. The status of DNA methylation on a susceptible gene may therefore be used to identify smokers who might be at risk for the development of COPD. Furthermore, this suggests a central role of DNA methylation in the regulation of inflammatory responses in smokers and in the pathogenesis of COPD.

1.4.3. Gene transcription

Inflammatory mediators play a crucial role in chronic inflammatory processes and appear to determine the nature of the inflammatory response by directing the selective recruitment and activation of inflammatory cells and their perpetuation within the lungs. In vitro studies using macrophage, alveolar, and bronchial epithelial cells, ROS have been shown to cause increased gene expression of inflammatory mediators, such as IL-1 and TNF-α. Direct or indirect oxidant stress to the airway epithelium and alveolar macrophages may also generate cytokines, such as TNF-α, which in turn can activate airway epithelial cells to induce pro-inflammatory genes, such as TNF-α, IL-8, IL-1, inducible NO synthase (iNOS), COX-2, intracellular adhesion molecule-1 (ICAM-1), IL-6, MIP-1, GM-CSF, stress response genes (HSP-27, -70, -90, HO-1) and antioxidant enzymes (γ-GCS, MnSOD and thioredoxin) [1]. Indeed, such responses have been observed in the BAL fluid of smokers [89,90]. The genes for these inflammatory mediators are regulated by redox-sensitive transcription factors, such as NF-κB and AP-1 [60,61]. Acetylation of histones has been associated with the transcription of a range of inflammatory mediators including IL-8 [62], eotaxin, IL-1β and GM-CSF [63], MIP-2 [64] and IL-6 [65]. Acetylation can occur specifically at the promoter sites of these genes as shown by chromatin immunoprecipitation (ChIP) assays for IL-8 [66], CYP1A1 [67], myeloperoxidase [68], COX-2 [69] and 15-lox-1 [70] gene promoters, indicating acetylation specificity.

1.4.4. Impact of oxidative stress on HDAC function

Oxidative stress and pro-inflammatory mediators have been suggested to influence histone acetylation and phosphorylation, via a mechanism dependent on the activation of the MAPK pathway [71–73]. This leads to increased transcription factor activity and subsequent recruitment of co-activators such as CBP and p300 with intrinsic HAT activity [65] (Fig. 3). Alternatively, disruption of HDAC activity by ROS or HDAC inhibitors, tilting the balance further towards increased HAT activity and DNA unwinding, could also facilitate transcription factor binding thereby enhancing pro-inflammatory gene expression. Both ROS and TNF-α can increase the activation of AP-1 and NF-κB, and regulate chromatin remodeling leading to IL-8 expression in lung cells [65,74,75]. Recently, Ito et al. have shown a role for histone acetylation and deacetylation in IL-1β-induced TNF-α release in alveolar macrophages derived from cigarette smokers [76]. They have also suggested that oxidants may play an important role in the modulation of HDAC and inflammatory cytokine gene transcription. They further demonstrated increased expression of p65 protein of NF-κB in bronchial epithelium of smokers and patients with COPD [77]. However, it is still unknown as to what happens to the NF-κB signaling pathway and chromatin remodeling in small airways and lung parenchyma of COPD patients. Our recent data indicated that cigarette smoke condensate increased the acetylation of histone 4 and this was also associated with decreased levels of HDAC-2 in alveolar epithelial cells [78]. We have also observed similar findings in vivo in the smoking rat model [79]. Moreover, we have shown that inhibiting HDACs alone resulted in enhanced activation of AP-1 and NF-κB and increased histone acetylation culminating in increased IL-8 release [74]. This observation is corroborated by previous studies, showing that acetylation of histone proteins is associated with increased binding of the transcription factor AP-1 and NF-κB [46,80]. IL-8 release was also augmented when trichostatin A, a histone deacetylase inhibitor, was combined with TNF-α or H_2O_2 and was similarly associated with increased NF-κB binding. It has also been shown that HDAC1 and 2 can interact directly with the p65 subunit of NF-κB to exert its corepressor function in the nucleus [66]. This suggests that inhibition of HDAC may not only promote NF-κB nuclear retention through its increased acetylation status [50], but that it prevents the intrinsic regulation of NF-κB activity through its association with HDAC co-repressor proteins, thereby augmenting pro-inflammatory gene transcription. In addition, NF-κB itself can be acetylated whilst in the nucleus via its interaction with CBP, which may lead to further augmentation of gene transcription (Fig. 4) [51]. Interestingly, Zhong and et al. have shown that in unstimulated cells the transcriptionally inactive nuclear p50 NF-κB subunit is complexed with HDAC1 preventing NF-κB dependent gene expression. Activation of cells with NF-κB-inducing agents leads to

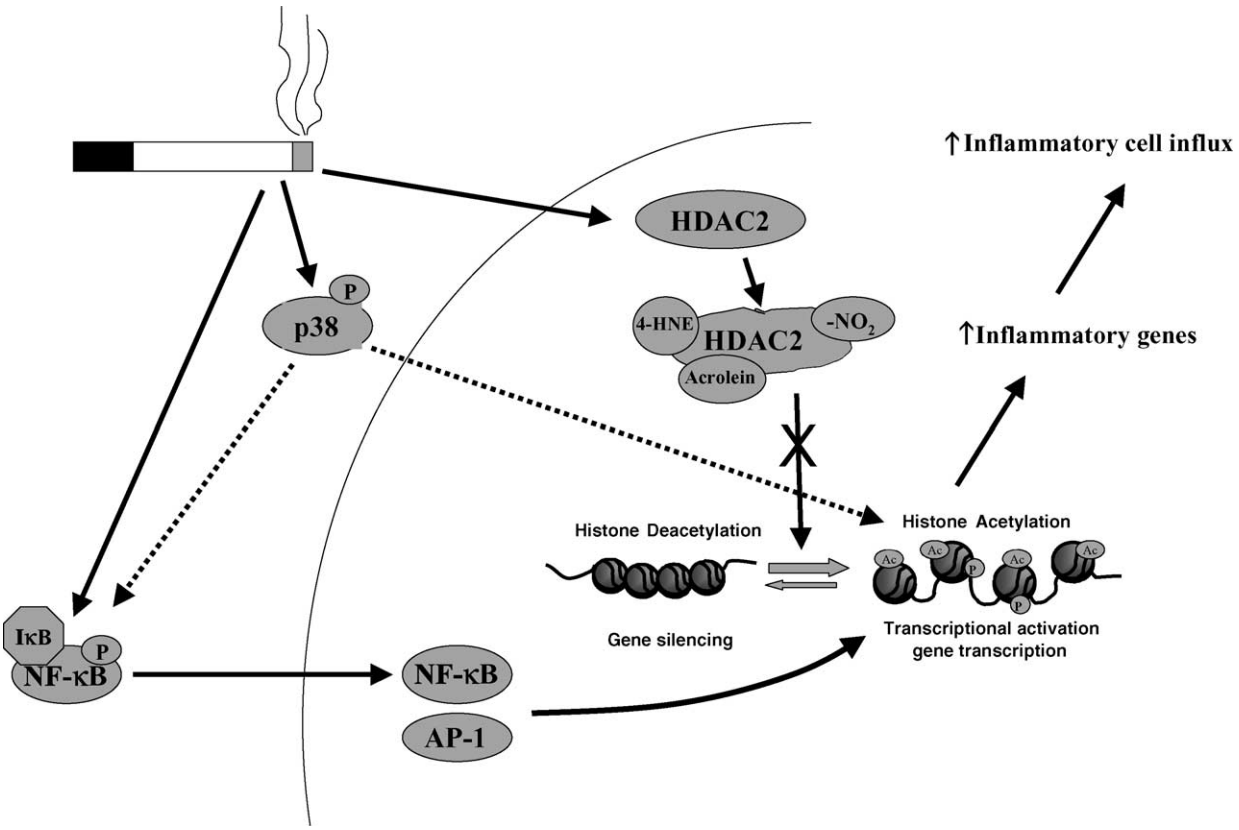

Fig. 5. The hypothesized mechanism of cigarette smoke-induced chromatin remodeling through a decrease in HDAC2 and increase in p38 MAPK phosphorylation resulting in an increase in histone 4 acetylation and histone 3 phospho-acetylation. The increased DNA binding of redox-sensitive transcription factors results in an increased transcription of specific pro-inflammatory genes marked out by phosphorylation of serine 10 on histone 3.

nuclear localization of active phosphorylated p65 that associates with CBP and displaces the p50-HDAC-1 complexes leading to gene transcription [81]. Moreover, under conditions of oxidative stress which leads to phosphorylation and activation of p65 NF-κB, only HDAC-2 and not HDAC-1 expression is decreased [79]. Consequently, very little HDAC-2 is able to bind to NF-κB, resulting in dysregulated control of NF-κB activation, increased histone acetylation and pro-inflammatory gene expression (Fig. 5).

It has been reported that IL-8 and IL-6 release is enhanced by HDAC inhibitors in intestinal epithelial cells and in murine fibrosarcoma L929sA cells [82,83]. Moreover, this can also enhance the effect of IL-1 or TNF-α treatments. Similarly, Rahman et al. have recently demonstrated that HDAC inhibitors (increasing the overall level of acetylation of the histone proteins) enhanced the levels of stromelysin-1 (matrix metalloproteinase-3) by augmenting histone acetylation by TNF-α or IL-1-stimulated mesenchymal cells [84]. Furthermore, it is reported that HDAC inhibitors enhanced pulmonary cells responsiveness to a subsequent stressor, such as H_2O_2 and TNF-α, leading to increased transcription factor DNA-binding and enhanced gene expression [85]. Similarly, IL-4 production from activated peripheral blood T cells was enhanced by the histone deacetylase inhibitor trichostatin A, and overexpression of HDACs 1–3 inhibited transcription driven by the IL-4 promoter in Jurkat T cells [86]. Co-transfection of the HAT-CBP potentiated IL-4 promoter activity suggesting that IL-4 expression is controlled at the level of gene transcription by chromatin remodeling. It has been shown that IL-4 gene expression was increased in lung cells obtained from smokers with COPD [87]. Thus, the gene expression of these pro-inflammatory mediators has important implications for inflammatory lung disease states where the HDAC enzyme is inactivated [76]. In these cases, ROS and TNF-α would lead to an augmented inflammatory response from the tissue. However, it is important to bear in mind that another HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA), inhibited release of key pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and IFN-γ, in monocytes/macrophages both in vitro and in vivo [88]. Thus, HDAC inhibitors may also exhibit anti-inflammatory properties through suppression of cytokine expression in a cell-specific manner. At present, however, the molecular mechanism for the inhibition of these cytokines by SAHA is not yet known.

1.4.5. Glucocorticoids and HDACs

Corticosteroids are potent anti-inflammatory agents. However, they appear ineffective in patients with COPD. It has been suggested that oxidative stress may have a role in the poor efficacy of corticosteroids in COPD. Glucocorticoid

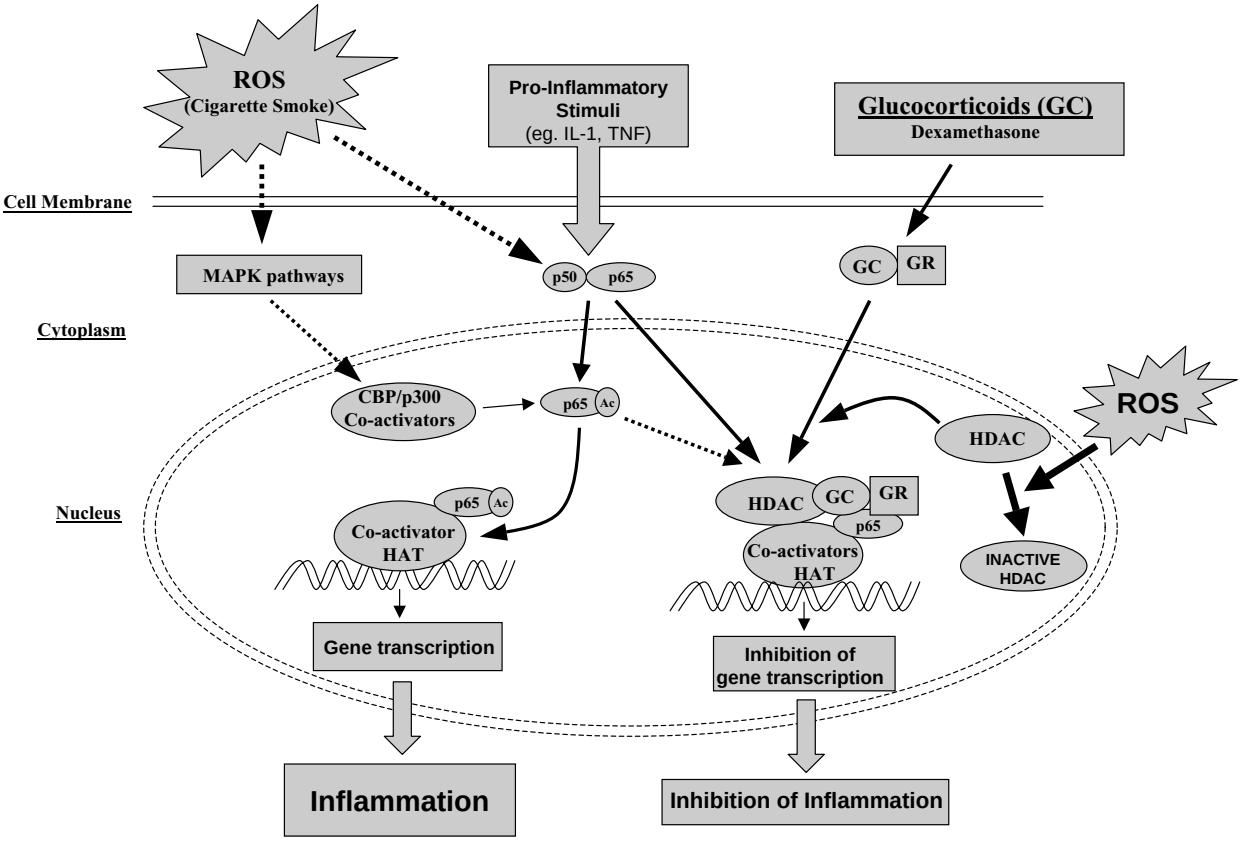

Fig. 6. Model illustrating the mechanism of corticosteroid (GC) action in suppressing pro-inflammatory gene expression and its impairment by oxidants. Direct interaction between co-activators (HAT), histone deacetylase and the glucocorticoid receptor (GR) results in repression of pro-inflammatory gene expression. HDAC forms a bridge with HAT to inhibit gene transcription. MAP kinase signaling pathways activated by oxidative stress can lead to p65 NF-κB subunit and histone acetylation. Moreover, when HDAC is inhibited by oxidants or the p65 NF-κB subunit is acetylated, steroids may not be able to recruit HDACs into the transcriptional complex to inhibit pro-inflammatory gene expression.

suppression of inflammatory genes requires recruitment of HDAC-2 to the transcription activation complex by the glucocorticoid receptor (Fig. 6) [49]. This results in deacetylation of histones and a decrease in inflammatory gene transcription. However, ROS and CSC inhibits HDAC-2 function. Consequently, a reduced level of HDAC-2 activity was found to be associated with an increased pro-inflammatory response and reduced responsiveness to glucocorticoids in alveolar macrophages obtained from smokers [76] as well as in vivo in rats exposed to cigarette smoke [79]. Similarly, the levels of HDAC activity and HDAC2 expression were found to be decreased in bronchial biopsies obtained from asthmatics [91]. This was partially restored in patients who received inhaled steroids, suggesting that steroids induced HDAC activity in asthmatics. In contrast COPD patients were unresponsive to corticosteroid therapy. In a recent study, Culpitt et al. have shown that cigarette smoke solution-stimulated release of IL-8 and GM-CSF was not inhibited by corticosteroids in alveolar macrophages obtained from patients with COPD compared to that of smokers [92]. They suggested that the lack of efficacy of corticosteroids in COPD might be due to steroid insensitivity of macrophages in the respiratory tract. Thus, the cigarette smoke/oxidant-mediated reduction in HDAC-2 levels in alveolar epithelial cells and macrophages will not only increase inflammatory gene expression but will also cause a decrease in glucocorticoid function in patients with COPD. This may be one of the potential reasons for the failure of glucocorticoids to function effectively in reducing inflammation in COPD (Fig. 6). Other possible mechanisms that could explain the glucocorticoid inefficacy in COPD include the acetylation of p65 by cigarette smoke-derived oxidants, the elevated levels of hepatocyte nuclear factor-6 or enhancement of macrophage migrating inhibitory factor which can antagonize/override glucocorticoid-stimulated gene transcription [50,93,94]. It is also interesting to note that certain other translocatory HDACs, such as HDAC3 and HDAC5 which shuttle between cytoplasm and the nucleus may also be important in regulating the transcriptional initiation complex by the glucocorticoid receptor. The signaling mechanisms involved in cigarette smoke-mediated chromatin remodeling and glucocorticoid insensitivity have only recently started to be unraveled. Initial findings would indicate that cigarette smoke ROS, RNS and lipid peroxidation products are responsible for post-translational modification and loss of HDAC-2 activity during inflammation (Fig. 7) [79,95]. Once the HDAC2 is modified it is then degraded by the ubiquity-proteosome pathway [96]. However, it is unclear yet which

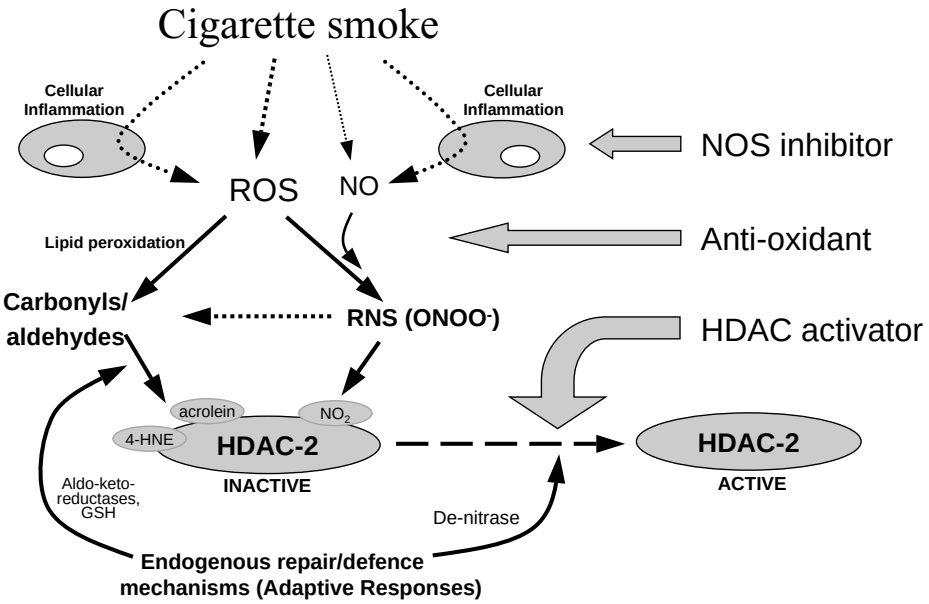

Fig. 7. Potential therapeutic intervention strategies to restore HDAC function. Cigarette smoke provides both direct and indirect ROS/RNS. This results in lipid peroxidation product formation along with peroxynitrate generation resulting in HDAC2 modification and inactivation. Endogenous repair/defence mechanisms which can potentially reverse or prevent these modifications become overwhelmed. Possible therapeutic intervention can be considered at several levels as illustrated. The net effect being to restore HDAC-2 function and thereby corticosteroid responsiveness.

modification (tyrosine nitration or lipid peroxidation product modification) is necessary to trigger degradation via this pathway. Furthermore, protective mechanisms, such as denitrases, are also present that have the potential to reverse these nitration modifications restoring HDAC activity [97]. Nevertheless, oxidative stress results in an imbalance between histone acetylation and deacetylation, which may account for the enhanced expression of inflammatory mediators leading to amplification of lung inflammation. This may serve as a potential mechanism for therapeutic intervention to ameliorate the chronic inflammatory response and loss of steroid efficacy which occurs in the development of smoking-induced chronic inflammatory lung diseases, such as COPD (Fig. 7).

1.5. Modulation of pro-inflammatory gene transcription by antioxidants, NOS inhibitors and HDAC upregulators

1.5.1. Thiol compounds

1.5.1.1. N-Acetyl-L-cysteine. NAC, a cysteine-donating reducing compound, acts as a cellular precursor of GSH and becomes de-acetylated in the gut to cysteine following oral administration. NAC may also reduce cysteine to cysteine, which is an important mechanism for intracellular GSH elevation in vivo in lungs [98]. It reduces disulfide bonds, but also has the potential to interact directly with oxidants. NAC is also used as a mucolytic agent (to reduce mucus viscosity and to improve mucociliary clearance). Pharmacological approaches, particularly with thiol antioxidants, such as NAC have been used in an attempt to enhance lung GSH in patients with COPD with varying success [98,99]. There have also been studies of patients with COPD where the administration of NAC has lead to a conflicting result [100]. A multi-centre study using NAC by metered dose inhalers in patients with chronic cough failed to show a positive effect on well being, sensation of dyspnoea, cough or lung function [101]. In contrast, Van Schooten et al. have reported that oral administration of NAC, 600 mg twice daily for a period of 6 months in a randomized, double-blind, placebo-controlled, Phase II chemoprevention trial, reduced various plasma and BAL fluid oxidative biomarkers in smokers [102]. NAC inhibited oxidant- and cytokine-mediated pro-inflammatory mediator release in alveolar epithelial cells (reviewed in 22).

1.5.1.2. N-Acystelyn (NAL). NAL a lysine salt of *N*-acetyl-L-cysteine, is a mucolytic and antioxidant thiol compound. The advantage of NAL over NAC is that it has a neutral pH solution, whereas NAC is acidic. Recently, a couple of studies have shown that NAC and NAL inhibited oxidant-mediated IL-8 release in A549 cells. This suggests that NAL, at least in vitro, had an anti-inflammatory effect in the systems studied [103,104]. Therefore, NAL may represent an interesting alternative approach to augment the antioxidant screen and thereby inhibit the oxidant driven inflammatory responses in vivo.

1.5.1.3. Glutathione peroxidase mimic. This is based on the approach that glutathione peroxidase can be manipulated

by small molecules with activity similar to this enzyme. Ebselen is a seleno-organic compound, an important element in the glutathione peroxidase catalysis of the reaction between GSH and ROS [105,106]. This increases the efficiency of GSH as an antioxidant, and can thus be used as a therapy against oxidative stress and inflammation. However, further studies are needed to validate the bioavailability of these compounds in lung inflammation.

1.5.1.4. Redox sensor molecules. There are other small redox molecules such as β-strand mimetic template MOL 294 and PNRI-299 which have been shown to inhibit NF-κB- and AP-1-mediated transcription and blocks allergic airway inflammation in a mouse asthma model [107]. The mechanism of inhibition is based on the reversible inhibition of redox sensor proteins (similar to redox effector factor-1). These redox compounds are novel and have been shown to reduce airway eosinophil infiltration, mucus hypersecretion, edema and cytokine release in this murine model. Similarly, a small molecular weight SOD mimetic (AEOL10150) has been shown to inhibit cigarette smoke-mediated inflammatory effects in vivo in a smoking rat model [108]. However, this group did not show whether this treatment also restored steroid sensitivity in their model system. This being of particular note, as we have shown that steroid sensitivity in vivo in the lung is lost in the smoking rat model [79].

1.5.1.5. Theophylline. Theophylline, a methyl xanthine, has been used for many years as a bronchodilator in patients with COPD. Concomitant therapy, theophylline plus corticosteroid (oral and inhaled) are given to these patients with varying success. Corticosteroids recruit HDACs and theophylline enhances HDAC activity in epithelial cells and macrophages thereby inhibiting the inflammation. However, only at low concentrations, below those given for bronchodilatory effects in the clinic, does theophylline appear to have an effect on HDAC both in vivo and in vitro [109].

1.5.1.6. Polyphenols. Curcumin (diferuloylmethane) is a naturally occurring flavonoid (polyphenol) present in the spice, turmeric, which has a long traditional use as a chemotherapeutic agent for many diseases. Curcumin is an active principle of the perennial herb *Curcuma longa* (commonly known turmeric). Turmeric has a long traditional use in the Orient for many ailments, particularly as an anti-inflammatory agent. Recent studies have reported that curcumin inhibits NF-κB expression/activation, cyclooxygenase (COX-2), heme oxygenase-1 (HO-1), cytokines and neutrophil recruitment in the lungs. Moreover, it has also been shown to protect against cigarette smoke-mediated oxidative stress [110]. Curcumin acts as an oxygen radical and hydroxyl radical scavenger, both which are formed by cigarette smoke. It also increases antioxidant glutathione levels by induction of γ-GCS and behaves as an anti-inflammatory agent through inhibition of NF-κB and IL-8 release in lung cells [111]. Resveratrol, a flavonoid found in red wine, is an effective inhibitor of inflammatory cytokine release from macrophages in COPD patients [112]. However, the bioavailability of the resveratrol orally is extremely low. Nevertheless, the anti-inflammatory property of resveratrol may be due to its ability to induce sirtuins, members of the class III HDAC family [113]. Clearly the molecular mechanisms responsible for the anti-inflammatory properties of these dietary polyphenols against cigarette smoke/oxidative stress-driven inflammation need further investigation.

2. Conclusions

There is now clear evidence for ROS/free radicals being important in the pathogenesis of various chronic inflammatory lung diseases, such as asthma and COPD, and lung cancer. ROS may be critical not only for the inflammatory response to cigarette smoke/environmental oxidants, through the activation of redox-sensitive transcription factors, DNA nicking, alteration in histone acetylation/deacetylation and hence pro-inflammatory gene expression; but may also be involved in the protective mechanisms against the effects of cigarette smoke by the induction of antioxidant/stress genes. Further understanding of the effects and roles of ROS in basic cellular functions, such as amplification of pro-inflammatory and immunological responses, signaling pathways, activation of transcription factors, chromatin modeling (histone acetylation and deacetylation) and gene expression, will provide important information regarding basic pathological processes contributing to chronic inflammatory lung diseases. Such knowledge would provide the basis for the development of novel therapeutic strategies to prevent or halt the progression of these diseases. Furthermore, as our understanding of gene expression/epigenetics/genomics increases, additional clinical targets and therapeutic strategies are likely to emerge.

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