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Oxidative stress, transcription factors and chromatin remodelling in lung inflammation

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Abstract

Oxidative stress has been implicated in the pathogenesis of several inflammatory lung disorders. Oxidants and inflammatory mediators such as tumour necrosis factor-α (TNF-α) activate transcription factors such as nuclear factor-kappaB (NF-κB) and activator protein-1 (AP-1) leading to the expression of pro-inflammatory genes. The expression of many genes, including those encoding pro-inflammatory mediators involves the remodelling of the chromatin structure provided by histone proteins. Histone acetylation causes the unwinding of chromatin structure therefore allowing transcription factor access to promoter sites. Nuclear histone acetylation is a reversible process, and is regulated by a group of acetyltransferases (HATs) which promote acetylation, and deacetylases (HDACs) which promote deacetylation. In addition, several co-activators, transcription factors and nuclear proteins also have histone acetyltransferase activity. Both TNF-α and the oxidant, hydrogen peroxide (H_2O_2) alter histone acetylation/deacetylation, and the activation of NF-κB and AP-1, leading to the release of the pro-inflammatory cytokine interleukin-8 (IL-8) in human alveolar epithelial cells (A549). Pharmacological inhibition of HDAC leads to the increased HAT activity, AP-1 and NF-κB activation, and IL-8 release by H_2O_2 or TNF-α treatments. This suggests that the remodelling of chromatin by histone acetylation plays a role in the oxidant-mediated pro-inflammatory responses in the lungs.

Keywords: Oxidant; TNF-α; GSH; AP-1; NF-κB; Interleukin-8; Histone acetylation; Deacetylases; Alveolar epithelial cells

1. Introduction

Airway inflammation is a characteristic of many lung disorders including asthma, chronic obstructive pulmonary disease (COPD), adult respiratory distress syndrome and idiopathic pulmonary fibrosis. All these diseases involve the recruitment of immune and inflammatory cells to the lungs. These cells are activated and produce mediators of inflammation including oxidants and cytokines, such as the pro-inflammatory cytokine tumour necrosis factor-α (TNF-α) [1,2].

Reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet -}$) and hydroxyl radical ($^{\bullet}OH$) are unstable molecules with unpaired electrons, capable of initiating oxidation. 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 (in air pollutants or cigarette smoke). The lung exists in a high-oxygen environment and, together with its large surface area and blood supply, is susceptible to injury mediated by ROS. Increased ROS production has been directly linked to oxidation of proteins, DNA, and lipids which may cause direct lung injury or induce a variety of cellular responses through the generation of secondary metabolically reactive species. ROS may alter or induce physiological events in the lung such as remodelling of extracellular matrix, apoptosis, mitochondrial respiration, cell proliferation, maintenance of surfactant and the antiprotease screen, alveolar repair and immune modulation [3,4].

The antioxidant glutathione (GSH) has been shown to be critical to the lungs' antioxidant defences, particularly in

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Abbreviations: A549, alveolar epithelial cells; AP-1, activator protein-1; ATF-2, activating factor-2; CBP, cyclic AMP response element binding (CREB)-binding protein; COPD, chronic obstructive pulmonary disease; EMSA, electrophoretic mobility shift assay; GSH, glutathione; H_2O_2, hydrogen peroxide; H4, histone 4; IL-8, interleukin-8; HDAC, histone deacetylase; HAT, histone acetyltransferase; MAPK, mitogen activated protein kinase; NF-κB, nuclear factor-kappaB; TSA, trichostatin A.

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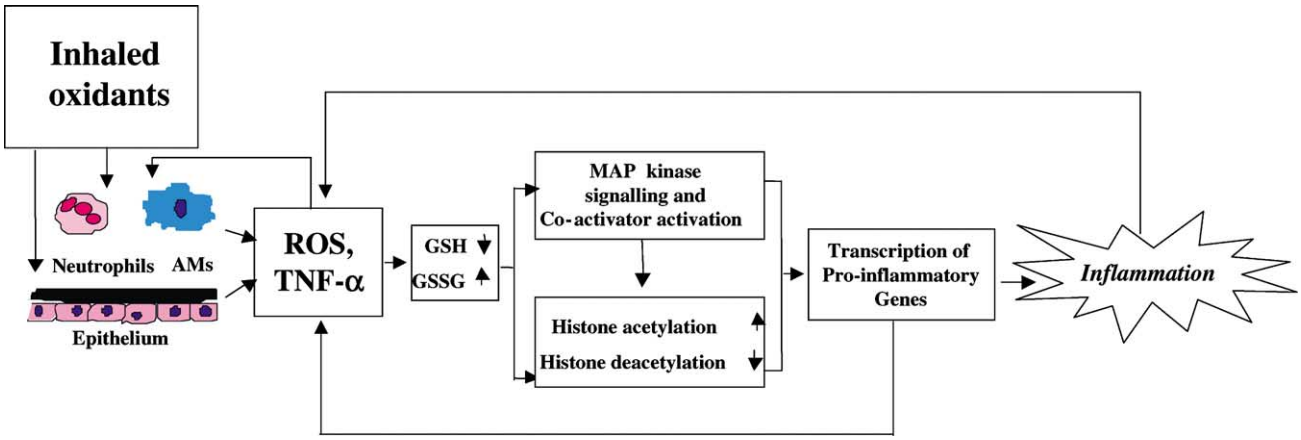

Fig. 1. Proposed mechanisms of oxidant-mediated lung inflammation. Inflammatory response is mediated by oxidants either inhaled and/or released by the activated neutrophils, alveolar macrophages and epithelial cells producing more oxidants and TNF-α leading to depletion/oxidation of the antioxidant, GSH. The oxidant/antioxidant imbalance signals for an increase in histone acetylation by the activation of the MAP kinase pathway. This leads to an increase in the transcription of inflammatory mediators, causing lung inflammation.

protecting airspace epithelium from oxidant injury and inflammation [1]. GSH redox homeostasis controls the level of ROS that are constantly formed during cellular metabolism. ROS and alterations in GSH levels are also implicated in the pathogenesis of several other autoimmune/inflammatory diseases [2]. Furthermore, increased levels of ROS have been implicated in initiating the lung inflammatory response through chromatin modelling (histone acetylation/deacetylation), the activation of transcription factors such as nuclear factor-kappaB (NF-κB) and activator protein-1 (AP-1) leading to gene expression of pro-inflammatory mediators [5,6] (Fig. 1). This review describes the TNF-α and hydrogen peroxide (H_2O_2) mediated redox regulation of NF-κB and AP-1 activation, and histone acetylation/deacetylation, leading to the induction of the pro-inflammatory gene interleukin-8 (IL-8).

1.1. Cell-derived ROS

A common feature of all inflammatory lung diseases is involvement of an inflammatory-immune response, characterised by activation of epithelial cells, and resident macrophages, and the recruitment and activation of neutrophils, eosinophils, monocytes, and lymphocytes. Inflammatory cells once recruited in the airspace become activated and generate ROS in response to a sufficient level of a secretagogue stimulus (threshold concentration). The activation of macrophages, neutrophils and eosinophils generates $O_2^{\bullet -}$, which is rapidly converted to H_2O_2 by superoxide dismutase (SOD), and $^{\bullet}OH$, formed non-enzymatically in the presence of Fe^{2+} as a secondary reaction. ROS and reactive nitrogen species (RNS) can also be generated intracellularly form several sources such as mitochondrial respiration, the NADPH oxidase system and xanthine/xanthine oxidase. It is proposed that ROS produced by phagocytes that have been recruited to sites of inflammation, are a major cause of the cell and tissue damage associated with many chronic inflammatory lung diseases such as asthma and COPD [2,7–9].

1.2. ROS, inflammatory mediators and activation of transcription factors

TNF-α is a pleiotrophic protein that mediates a multitude of inflammatory events in the lung [1]. The induction of inflammatory mediators can be regulated by the activation of redox-sensitive transcription factors AP-1 (c-*fos*/c-*jun*) and NF-κB stimulated in response to oxidants and TNF-α [5]. ROS and cellular redox status, particularly intracellular thiol status can be directly involved in the activation of AP-1 and NF-κB, and signal transduction [10–12]. TNF-α increases AP-1 binding *via* the mitogen activated protein kinase (MAPK) signalling pathway, and activates NF-κB *via* the inhibitory kappa-B (Iκ-B) kinase pathway [10,13,14]. Binding of NF-κB and AP-1 leads to the transcription of genes for several inflammatory mediators and chemokines, including IL-8 [13,15,16].

Both environmental or inflammatory cell-derived ROS can lead to the activation and phosphorylation of the MAPK family, including extracellular signal regulated kinase (ERK), c-*jun* N-terminal kinase (JNK) and p38 kinase as well as the phosphoinositide 3 signalling protein (PI-3K) *via* cysteine rich domains. In addition, the sphingomyelinase–ceramide pathway is also stimulated in response to ROS [5,6,17]. Activation of members of the MAPK family leads to the transactivation of transcription factors such as c-*jun*, activator transcription factor-2 (ATF-2), CBP and Elk-1 [17–19]. This eventually culminates in chromatin remodelling and expression of genes regulating a battery of distinct pro-inflammatory genes involved in several cellular events including apoptosis, proliferation, transformation and differentiation.

The exact intracellular molecular signalling mechanism of ROS action has not been completely characterised.

Redox sensitive molecular targets usually contain highly conserved cysteine residues, and their oxidation, nitration, and formation of disulphide links are crucial events in oxidant/redox signalling. It is hypothesised that oxidation of those sulphide groups in signalling proteins causes structural modifications, resulting in the exposure of active sites and protein activation. Such molecular targets include transcription factors (NF-κB, AP-1), signalling molecules such as ras/rac or JNK, protein tyrosine phosphatases and $p21^{ras}$ [17,18,20]. Thiol molecules such as intracellular GSH and thioredoxin are of central importance in the regulated control of such redox signalling pathways, by reducing disulphide bridges or oxidised cysteine residues [21].

Recent studies have shown that in response to TNF-α and lipopolysaccharide (LPS), which are relevant stimuli for the inflammatory response in lung diseases, airway epithelial cells can concurrently produce increased amounts of intracellular ROS and RNS [22]. This intracellular production of oxidants and the subsequent changes in intracellular GSH redox status is important in the molecular signalling events controlling the expression of genes for inflammatory mediators [5,22]. The signalling pathways and activation of transcription factors in response to ROS are the subject of rigorous investigation.

It has been suggested that oxidant generating systems and pro-inflammatory mediators influence histone acetylation/phosphorylation *via* a mechanism dependent on the activation of the MAPK pathway [23–25]. Oxidative stress induced by H_2O_2 and TNF-α increases the activation of AP-1 and NF-κB, and may regulate chromatin remodelling leading to IL-8 expression. However, the effect of TNF-α and H_2O_2 on histone acetylation/deacetylation have not been studied in mammalian cells. It is also unknown how the modulation of nuclear histone acetylation/deacetylation affects IL-8 release and NF-κB/AP-1 binding.

1.3. Chromatin remodelling

Gene transactivation is controlled by multimeric complexes of transcriptional co-activators and co-repressors which bind to consensus sites within gene promoters and regulate transcription [26]. 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 [27,28]. 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 [29]. The basic unit of chromatin, the nucleosome, consists of 146 base pairs of DNA wrapped around a core histone structure consisting of an H_3/H_4 tetramer and two H_2A/H_2B dimers. Tightly bound DNA around a nucleosome core suppresses gene transcription by decreasing the accessibility of transcription factors, such as NF-κB and AP-1 to gene promoters. Acetylation of lysine residues in the N-terminal tails of the core histone proteins in presence of acetyl CoA results in uncoiling of the DNA (chromatin relaxation), allowing increased accessibility for transcription factor binding [29]. Acetylation of lysine (K) residues on the N-terminal tails of histone 4 (H4; K5, K8, K12, K16) is thought to be directly related in the regulation of gene transcription [30,31]. There are several distinct lysine residues in each histone protein available for acetylation. The acetylation of distinct histones or different combination of lysine residues may serve as target sites of regulation, altering the chromatin conformation in such a way as to allow transcription factors to upregulate distinct sets of genes. Thus the acetylation of core histones is critical in the remodelling of chromatin and therefore gene expression, and is involved for transcription to occur. However, core histones may also be modified by phosphorylation, methylation, ADP-ribosylation, or ubiquitination of a specific amino acid residue [19].

Histone acetylation is reversible and is regulated by a group of acetyltransferases (HATs) which promote acetylation, and deacetylases (HDACs) which promote deacetylation [32]. The family of HDAC enzymes consists of 10 distinct deacetylases. HDACs remove the acetyl moieties from the ε-acetamido groups of lysine residues of histones (restoration of positive charges) causing rewinding/condensation of DNA associated with displacement of transcription factors from their cognitive DNA binding sites leading to silencing of gene transcription. HDACs associate in multimeric complexes which serve to direct these proteins to specific promoter sites. HDACs represent a super family of molecules sharing a 390-amino acid region of homology known as the deacetylase core [33]. Study using a powerful technique of X-ray crystallography has shown that the active site of these enzymes is formed by a tubular pocket, a zinc-binding site, and two sets of aspartic acid and histidine residues [34], all of which are conserved across all HDACs. HDACs are found complexed to co-repressor molecules like Sin3, N-CoR, and SMRT [33]. Recently, the role of HDACs (1 and 2) has been shown in the regulation of cell proliferation and corticosteroid-mediated inhibition of pro-inflammatory mediators [35,36]. Several distinct HDACs are now recognised, and these are differentially expressed and regulated in different cell types. These HDACs may well be involved in various biological events, and may also contribute to the responsiveness to corticosteroids between different stimuli, genes and cell types.

1.4. Histone acetyltransferase (HAT)/co-activators

HAT promotes acetylation though the transfer of an acetyl group from acetyl CoA onto the lysine tails of histone, thereby inducing a conformational change of histone bound DNA. Intriguingly, several HATs have been

shown an ability to acetylate non-histone protein substrates (e.g. transcription factors) *in vitro* as well, suggesting the possibility that internal lysine acetylation of multiple proteins exists as a rapid and reversible regulatory mechanism much like protein phophorylation. Recent evidence has shown that histone acetylation and deacetylation can play a causative role in the regulation of gene transcription.

The nuclear receptor co-activators, steroid receptor co-activator 1 (SRC-1), cyclic AMP response element binding (CREB)-binding protein (CBP)/adenoviral protein E1A (p300) protein, CBP/p300 associated factor (P/CAF), and ATF-2, all possess intrinsic HAT activity [37–39]. Of these, the master phosphoprotein CBP/p300 and ATF-2, which are regulated by MAP kinase pathways, are vital for the co-activation of several transcription factors including NF-κB and AP-1 in the transcription machinery [37,38,40]. These multimeric activation complexes act with RNA polymerase II to initiate transcription [41,42]. Thus, it is likely that histone acetylation of histone 3 (H3)/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 [38,41–44], although the precise molecular signalling mechanisms are still not fully understood.

Pro-inflammatory gene transcription regulation is a multifaceted process which requires a combination of nuclear factors and co-activators for maximal gene transcription to occur. For example, the TNF-α-induced activation of interleukin-6 (IL-6) transcription is reliant on the activation of NF-κB, and AP-1, and recruitment of co-activators such as CBP and p300 [45]. Moreover, the CBP/p300 co-activator proteins involved in NF-κB (p65), and other transcription factor, complexes are associated with chromatin remodelling which facilitates gene transcription *via* the dislocation of repressor molecules which have been speculated to, in turn, inhibit histone deacetylase (HDAC) enzymes [45,46]. Differential activation of gene expression by transcription factors and co-activator proteins is likely to occur as a result of specific binding sites for different transcription factors which affect the HAT activity of the co-activators [46]. Therefore, the signal transduction pathways which activate transcription factors [5,13,47], may be responsible for stimulus specific chromatin remodelling, leading to gene transcription, *via* co-activator HAT activity.

1.5. Role of ROS in chromatin remodelling

1.5.1. Role of HAT and NF-κB in pro-inflammatory gene transcription

The role of the nucleosome remodelling in the control of gene transcription co-activator and transcription factor access to the target promoter sites of genes is increasingly viewed as an important regulatory mechanism for the transcriptional activation of genes. Levels of histone acetylation have been directly related to the levels of gene transcription [48]. Oxidative stress and pro-inflammatory mediators have been suggested to influence histone acetylation and phosphorylation by ADP-ribosylation, *via* a mechanism dependent on the activation of MAPK pathway [23,25]. Recently, it has been shown that both H_2O_2 and TNF-α caused an increase in HAT activity in alveolar epithelial cells (A549) [49,50]. The exact signalling mechanism of increased histone acetylation in response to these agents is not clear. It has been reported that oxidants and TNF-α activate MAPK pathways, specifically ERK and JNK, as well as redox-sensitive signalling pathways that may regulate co-activators, such as ATF-2 and CBP [24,38,40–42]. However, it has been recently shown that p38 kinase is also involved in H3 phosphorylation at serine 10 followed by acetylation at lysine 14 (referred to as phosphoacetylation) by LPS stimulation of dentritic cells [44].

Histone acetylation has been reported in response to cytokines (IL-1, GM-CSF), oxidants and cigarette smoke [24,49,50], stimuli that activate cells by signal transduction and oxidative stress mechanisms. Miyata *et al.* [24] have shown that H3 and H4 are acetylated in murine epithelial cells in response to GM-CSF at the myeloperoxidase gene promoter site, a response which was dependent upon MAPK activation. Similarly, Berghe *et al.* [45] have demonstrated the involvement of ERK and p38 protein kinases for IL-6 expression in response to TNF-α. Therefore the acetylation of H3 and H4 is important in the signal transduction induced activation of target genes in response to stimuli. As MAPK activation has been associated with histone acetylation [24,44], the cell signalling pathway may play a role in the histone mediated pro-inflammatory effects.

Increased histone acetylation occurs following stimulation with serum, platelet-derived growth factor, or the protein inhibitor, cycloheximide, accompanied by the induction of the immediate early response genes, c-*fos* and c-*myc* in murine fibroblasts [51]. Shankaranarayanan *et al.* [52] have shown that acetylation of H3 and STAT6 by CBP/p300 is associated with 15-lipoxygenase-1 gene expression induced by IL-4 in A549 lung epithelial cells. In this model, deacetylation of H3 enabled promoter activation leading to promoter-mediated gene expression thus outlining the importance of specific acetylation in facilitation of gene expression in the cell type. Ito *et al.* [36] demonstrated in lung type II cells that IL-1β enhanced both the acetylation of H4 at lysines 8 and 12 and GM-CSF. However, phosphorylation/phosphoacetylation of H3 has been shown upon LPS treatment of human dendritic cells leading to induction of IL-6, IL-8, IL-12 and macrophage chemoattractant protein-1 without any change in macrophage inflammatory protein-1α and TNF-α expression by chromatin immunoprecipitation assay (ChIP) [44]. These studies suggest stimulus and cell specific phosphorylation/acetylation of histone proteins.

ROS and TNF-α increase the activation of AP-1 and NF-κB, and regulate chromatin remodelling leading to IL-8

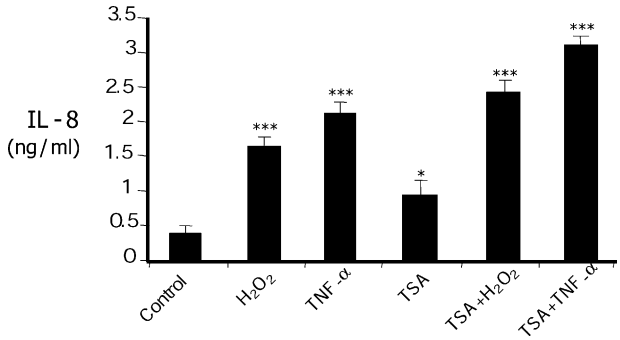

Fig. 2. The effect of TNF-α (10 ng/mL), H_2O_2 (100 mM) and TSA (100 ng/mL) for 20 hr alone or in combinations on IL-8 release in A549 epithelial cells. Cells were pretreated with trichostatin A (TSA) 6 hr before the co-treatment of TNF-α or H_2O_2. (∗) denotes $P < 0.05$ and (∗∗∗) denotes $P < 0.001$, compared to control values.

expression in lung cells [16,53] (Fig. 2). IL-8 is a chemokine released during inflammation and is important in the recruitment and activation of immune and inflammatory cells. Recently, Ito *et al.* [49] have shown a role for histone acetylation and deacetylation in IL-1β-induced TNF-α release in alveolar macrophages derived from cigarette smokers. They have also suggested that oxidants may play an important role in the modulation of HDAC and inflammatory cytokine gene transcription. It has been reported that histone acetylation plays a role in IL-8 and IL-6 gene expression [45,54,55], whereas IL-8 gene silencing is associated with increased HDAC activity [45,55]. The oxidant and TNF-α-stimulated expression of IL-6/IL-8 is regulated by NF-κB by a mechanism that involves its intrinsic HAT activity [45,54]. Hence, histone acetylation may be a regulatory mechanism for the induction of immediate-early response genes and pro-inflammatory mediators and this may be a prerequisite for the pro-inflammatory effects induced by oxidative stress.

Our laboratory has shown that oxidants increase the acetylation of H4, which is associated with a decrease in HDAC-2 levels in A549 [56]. Furthermore, inhibition of HDACs alone resulted in enhanced activation of AP-1 and

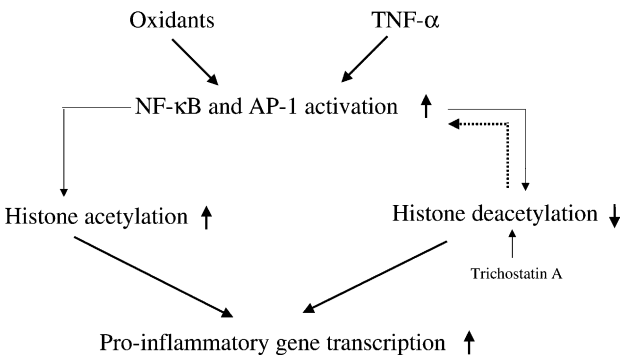

Fig. 3. Schematic diagram showing ROS and TNF-α-mediated activation of NF-κB and AP-1 leading to alteration in histone acetylation/deacetylation and increased pro-inflammatory gene transcription.

NF-κB, and increased histone acetylation culminating in increased IL-8 release (Fig. 3). TNF-α, H_2O_2 and TSA increased the NF-κB activation in human A549. This suggests that HDAC inhibition is sufficient to activate NF-κB in these cells. It is known that p65, a component of the NF-κB transcription factor, has intrinsic HAT activity and transactivation of p65 is independent of nuclear translocation [55,57,58]. It has been recently shown that HDAC-1 can interact directly with the p65 subunit of NF-κB to exert its co-repressor function in the nucleus [55]. Therefore, NF-κB interaction with HDAC (1 and 2) proteins may be a further mechanism whereby NF-κB can regulate transcription [55]. HDACs may be prevented from binding to nuclear p65 by oxidants leading to enhanced p65 acetylation/phosphorylation resulting in IL-8 gene expression. This pathway may not require the classic NF-κB/Iκ-B kinase pathway [44,55,59].

We found that IL-8 release was also augmented when TSA (HDAC inhibitor) was combined with TNF-α or H_2O_2 and found to be associated with NF-κB activation. This suggests that inhibition of HDAC allows NF-κB to be actively retained in the nucleus triggering augmented TNF-α or H_2O_2-mediated HAT activity leading to gene transcription. In addition, NF-κB itself is acetylated, whilst in the nucleus thereby enhancing and prolonging gene transcription [60]. Once the 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.* [61], who have shown dual waves (two temporally distinct phases) of recruitment of NF-κB to target promoters by LPS stimulation in Raw 264.7 murine macrophage cell line. 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, Iκ-B-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 genes promoter occurs after nuclear entry and is preceded by the formation of an initial transcription factors complex that directs the hyperacetylation of the promoter and makes it accessible to NF-κB [39]. This shows the selectivity of stimulus-specific NF-κB-mediated histone acetylation leading to a subset of gene transcription. Furthermore, accessibility of NF-κB sites contained in different promoters may well be regulated by the differentiative or developmental state of the cell/tissue and therefore it may be possible that different mechanisms may regulate accessibility in distinct types of differentiated cells (Natoli G, personal communications).

Our laboratory has also shown that H_2O_2, TNF-α and HDAC inhibitor treatments all increased AP-1 binding, suggesting that inhibition of HDAC may activate MAPK pathways such as stress activated signal transduction pathways by JNK leading to AP-1 activation [11,13]. Ng *et al.* [43] showed that binding of c-*Fos*/c-*Jun* heterodimer into

an acetylated nucleosome resulted in complete disruption of the nucleosome structure and facilitated the subsequent binding of AP-1 transcription factor, leading to increased gene transcription. However, it was shown that co-incubation of H_2O_2 and TNF-α with TSA resulted in increased AP-1 activation and binding, but this was not significantly greater than H_2O_2 and TNF-α alone. The explanation of this effect is not known but it may be possible that H_2O_2 and TNF-α at the concentrations used induced maximum binding of AP-1 to DNA.

1.6. Role of HDACs in pro-inflammatory gene transcription

Inhibition of HDAC activity by ROS or TSA are known to enhance the expression of the pro-inflammatory mediators, such as ICAM-1, IL-8, IL-6, TNF-α, IL-1β, monocyte chemoattractant protein-1, matrix metalloproteinases and heat shock proteins in various cells [45,52,62–65]. Most of these cytokines are further enhanced by IL-1 or TNF-α co-treatments [45,49,55]. Pender *et al.* [62] 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. Inhibition of HDAC in tumour cell lines leads to specific chromatin acetylation and alteration of cell cycle proteins, including upregulation of $p21^{waf1/cip1}$ and cyclin E [35]. Moreover, our laboratory has shown that HDAC inhibitors enhances pulmonary cell responsiveness to a subsequent stressor, such as H_2O_2 and TNF-α, leading to increased transcription factor DNA-binding and enhanced IL-8 gene expression [56]. This is supported by the observation that TNF-α induces hyperacetylation of the IL-8 promoter [55]. This has an implication in inflammatory lung disease states where the HDAC enzyme is inactivated [49]. In these cases, ROS and TNF-α would lead to an augmented inflammatory response from the tissue [14].

It has been shown that glucocorticoid suppression of inflammatory genes requires recruitment of HDAC-2 to the transcription activation complex by the glucocorticoid receptor [36]. Glucocorticoids such as dexamethasone has been shown to target acetylation on H4 lysines K5 and K16, disrupt IL-1β stimulated K8 and K12 acetylation and inhibit GM-CSF induction [36]. This results in deacetylation of histones and a decrease in inflammatory gene transcription. The reduced level of HDAC-2 was associated with increased pro-inflammatory response and reduced responsiveness to glucocorticoids in alveolar macrophages obtained from smokers [49]. Our preliminary data also suggested that H_2O_2 and TNF-α-mediated induction of IL-8 was partially blocked by dexamethasone when the HDAC activity as inhibited by TSA in A549 (unpublished data). The oxidant-mediated reduction in HDAC-2 levels in A549 not only increases inflammatory gene expression but also causes a decrease in glucocorticoid function. This may be one of the potential reasons for the failure of glucocorticoids to function effectively in reducing inflammation in COPD. The signalling mechanisms involved in the cigarette smoke-mediated chromatin remodelling and glucocorticoid insensitivity are currently unknown. It may be possible that cigarette smoke-mediated formation of potential lipid peroxidation products/oxidants is responsible for oxidation/nitrosylation/phosphorylation of HDACs (1,2,5) during inflammation. 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 (Fig. 4). This may serve as a potential mechanism for therapeutic intervention to ameliorate the chronic inflammatory response which occurs in the development of oxidant-induced chronic inflammatory lung disease such as COPD and asthmatic smokers.

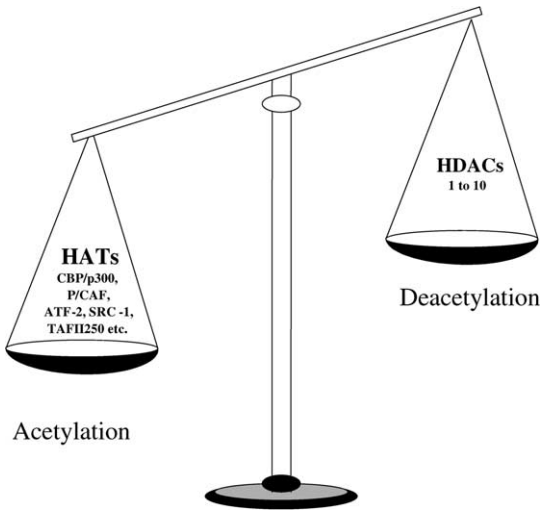

Fig. 4. HAT and deacetylase imbalance in inflammation. In inflammation, the balance appears to be tipped in favour of increased acetylation, either because of excessive release of ROS, or inflammatory mediators leading to amplification of the pro-inflammatory effects.

2. Conclusions

H_2O_2 and TNF-α modulate the intrinsic activity of HAT (presumably by modulation of HAT co-activators) leading to increased acetylation of histone proteins in A549. Inhibition of HDACs promoted acetylation of histone proteins (H4), which was associated with a decrease in GSH levels. HDAC inhibition was also related to an increase in NF-κB and AP-1 DNA binding and activation, as well as IL-8 release in A549. Thus, oxidant stress may result in an imbalance between histone acetylation and deacetylation, which may amplify lung inflammation. This may be a potential mechanism for the chronic inflammatory response which occurs in the development of chronic

inflammatory lung diseases. In addition, targeted acetylation of histone and/or increased deacetylation may represent a potential novel therapeutic intervention in inflammation. Further understanding of the effects and roles of ROS in basic cellular functions as amplification of pro-inflammatory and immunological responses, signalling pathways, activation of transcription factors (NF-κB and AP-1), chromatin modelling (histone acetylation and deacetylation), and gene expression will provide important information regarding basic pathological processes contributing to chronic inflammatory diseases.

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