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Forum Review

Redox Regulation of Histone Deacetylases and Glucocorticoid-Mediated Inhibition of the Inflammatory Response

IAN M. ADCOCK, BORJA COSIO, LOUKIA TSAPROUNI,
PETER J. BARNES and KAZUHIRO ITO

ABSTRACT

Gene expression, at least in part, is regulated by changes in histone acetylation status induced by activation of the proinflammatory redox-sensitive transcription factors activator protein-1 (AP-1) and nuclear factor-κB (NF-κB). Hyperacetylated histone is associated with open actively transcribed DNA and enhanced inflammatory gene expression. In contrast, hypoacetylated histone is linked to a closed repressed DNA state and a lack of gene expression. The degree of inflammatory gene expression is a result of a balance between histone acetylation and histone deacetylation. One of the major mechanisms of glucocorticoid function is to recruit histone deacetylase enzymes to the site of active gene expression, thus reducing inflammation. Oxidative stress can enhance inflammatory gene expression by further stimulating AP-1- and NF-κB-mediated gene expression and elevating histone acetylation. In addition, oxidants can reduce glucocorticoid function by attenuating histone deacetylase activity and expression. Thus, oxidant stress, acting through changes in chromatin structure, can enhance inflammation and induce a state of relative glucocorticoid insensitivity. This may account for the lack of glucocorticoid sensitivity in patients with chronic obstructive pulmonary disease. Antioxidants should reduce the inflammation and restore glucocorticoid sensitivity in these subjects. *Antioxid. Redox Signal.* 7, 144–152.

CHROMATIN REMODELING

ALTERATIONS IN THE STRUCTURE of chromatin are critical to the regulation of gene expression (73). DNA is tightly compacted around a protein core. This chromatin structure is composed of nucleosomes, which are particles consisting of ~146-bp DNA associated with an octamer of two molecules each of core histone proteins (H2A, H2B, H3, and H4). Expression and repression of genes are associated with alterations in chromatin structure by enzymatic modification of core histones (73). In the resting cell, DNA is tightly compacted around these basic core histones, excluding the binding of large protein complexes such as RNA polymerase II, which activates the formation of messenger RNA. This conformation of the chromatin structure is described as closed and is associated with suppression of gene expression (73) (Fig. 1).

The irregular 30-nm chromatin fiber is stabilized and further compacted by interactions between nucleosomes and linker histones such as H1. Histone H1 has long been regarded as a general repressor of transcription, but there are now indications that phosphorylation of histone H1 may play an essential role in activation of gene transcription (78). In this newer model, histone H1 acts as a "gate" to nucleosomal DNA, and histone H1 removal is needed for transcription factors to bind DNA, thus making nucleosomal DNA accessible to further transcription-factor binding and transcription (78).

Histone acetylation and gene transcription

Specific residues (lysines, arginines, and serines) within the N-terminal tails of histones are capable of being posttranslationally modified by acetylation, methylation, ubiquitination, or phosphorylation, all of which have been implicated in the regulation of gene expression (73). Acetylation of the ϵ-group on lysines reduces the charge of the histone residue and subsequently releases the tightly wound DNA, allowing the recruitment of further large protein complexes (73). A breakthrough in the discovery of the role of histone acetylation was

Thoracic Medicine, National Heart and Lung Institute, Imperial College London, London, U.K.

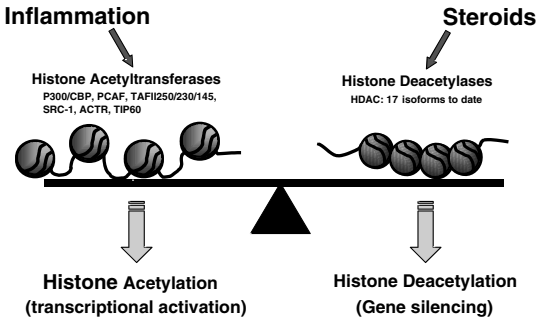

FIG. 1. Gene activation and repression are regulated by acetylation of core histones. Histone acetylation is mediated by coactivators, which have intrinsic histone acetyltransferase (HAT) activity, whereas repression is induced by histone deacetylases (HDACs), which reverse this acetylation. Numerous HATs, exist including CREB binding protein (CBP), p300/CBP-associated factor (PCAF), steroid receptor coactivator-1 (SRC-1), nuclear receptor coactivator-3 (NCOA3), and TAT interactive protein 60 (TIP60). HDACs are classified into three classes according to homology with yeast HDACs.

the demonstration that transcriptional coactivators such as CREB binding protein (CBP) and p300/CBP-associated factor PCAF) have intrinsic histone acetyltransferase (HAT) activity, which is activated by the binding of transcription factors (35, 73).

Increased gene transcription is therefore associated with an increase in histone acetylation, whereas hypoacetylation is correlated with reduced transcription or gene silencing (73). Histone acetylation is an active process whereby small changes in the activity of HATs or histone deacetylases (HDACs) can markedly affect the overall histone acetylase activity associated with inflammatory genes (73) (Fig. 1). Importantly, these changes in histone acetylation appear to be targeted toward regions of DNA associated with specific activator sites within the regulatory regions of induced inflammatory genes (73), although a global loosening of histone structure has also been proposed (75).

Histone deacetylation

Repression of genes is associated with the reversal of histone acetylation, or histone deacetylation, a process controlled by HDACs (20). The number of known HDACs is growing; so far, at least 17 mammalian forms have been identified (20). These HDACs are categorized into three classes according to homology with yeast HDACs. Interestingly, the regulation of at least some class 2 HDACs is directly linked to cellular signaling networks (22). Deacetylation of histones increases the winding of DNA around histone residues, resulting in a dense chromatin structure and reduced access of transcription factors to their binding sites, thereby leading to repressed transcription of inflammatory genes (73) (Fig. 1).

However, the simple model described above does not tell the full story. Under resting conditions, less than half of the potential lysine residues available for acetylation are in fact acetylated, and these residues have a rapid turnover (75). This situation suggests that even small changes above or below the resting level are enough to lead to an activated chromatin state. Furthermore, this model predicts that changes in the "histone code" (36) will be translated into downstream events extremely rapidly (75).

THE INFLAMMATORY BASIS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

COPD is among the most common diseases in the world, with an alarming increase in global prevalence (6, 47). COPD involves airway obstruction and chronic inflammation of the respiratory tract, with the increased expression of multiple inflammatory proteins. Most of these proteins are regulated at the transcriptional level, suggesting that in COPD transcription factors may play an important role (6, 9). Nuclear factor-κB (NF-κB) and activator protein-1 (AP-1) regulate many of the genes that are abnormally expressed in COPD, and we have shown that NF-κB is activated in the airways and alveolar macrophages of patients with COPD (21).

The inflammation in COPD, however, is markedly different from that seen in asthma in terms of the inflammatory cells involved, the mediators released, and the inflammatory effects (6, 7). A particularly striking difference between these two diseases is the response to corticosteroids; airway inflammation in asthma is suppressed by low doses of inhaled corticosteroids in most patients, whereas in COPD high doses of inhaled or even oral corticosteroids fail to reduce inflammatory cells, cytokines, or proteases and have no significant effect on lung function (28, 39, 54). Alveolar macrophages play a critical role in orchestrating the chronic inflammation in COPD through the release of proteases such as matrix metalloproteinase-9, inflammatory cytokines such as tumor necrosis factor-α (TNF-α), and chemokines such as interleukin (IL)-8 and macrophage inflammatory protein (MIP)-1α that attract neutrophils into the airways. Bronchoalveolar lavage (BAL) macrophages isolated from patients with COPD have a marked reduction in the inhibitory effect of dexamethasone on IL-8 and MIP-1α secretion, compared with normal subjects and asthmatic patients (16). This suggests that there might be an *active* mechanism of corticosteroid resistance in patients with COPD.

Oxidative stress and COPD

The lung is exposed to high levels of oxidant stress generated both endogenously, as a result of metabolism and/or the presence of inflammatory cells such as granulocytes, or from the environment, *e.g.*, cigarette smoke and ozone. Activation of macrophages, neutrophils, and eosinophils generates superoxide anion ($O_2^{\bullet -}$), which is rapidly converted to H_2O_2 by superoxide dismutase (SOD). A by-product of this reaction is hydroxyl radicals ($^{\bullet}OH$). These molecules ($O_2^{\bullet -}$ and $^{\bullet}OH$) and similar moieties are unstable molecules that rapidly gain an electron from the oxidizing substrates, thereby losing their unpaired electrons. These molecules are part of a collection of radical and nonradical species (*e.g.*, H_2O_2 and HOCl), which are also oxidizing agents and are known as reactive oxygen species (ROS) (23, 57). In the presence of nitric oxide (NO), elevated in many inflammatory conditions, ROS can also pro-

duce reactive nitrogen species (RNS), including peroxynitrite and 3-nitrotyrosine. To counteract the high oxidant load present, the lung and airways express high levels of antioxidants such as glutathione (GSH) and thioredoxin (23, 57).

ROS-sensitive target proteins generally have highly conserved cysteine residues that are potential targets for oxidation, nitration/nitrosylation, and cross-linking. In addition, the role of tyrosine nitration in ROS/RNS-mediated signaling is also becoming apparent (56). Increased markers of oxidative stress have been reported in sputum, breath, and lungs of patients with COPD, and these reflect elevated levels of ROS produced in the airways (41, 53). These markers include 8-isoprostane, H_2O_2, peroxynitrite, and 3-nitrotyrosine (41, 53) and the lipid peroxidation products acrolein, ethane, 4-hydroxy-2-nonenal, and isoprostanes (48).

NF-κB

Although numerous different pathways are activated during the inflammatory response, NF-κB is thought to be of paramount importance in asthmatic and COPD inflammation because it is activated by all the stimuli considered important (5). NF-κB is activated by numerous extracellular stimuli, including cytokines such as TNF-α and IL-1β, viruses and environmental particles [particulate matter ≤10 μm in diameter (PM10s)] and oxidative stress (5, 26). NF-κB is ubiquitously expressed within cells, and it not only controls induction of inflammatory genes in its own right, but also enhances the activity of other cell- and signal-specific transcription factors (5, 11, 51). NF-κB is a redox-sensitive transcription factor whose activity is regulated by changes in the oxidant/antioxidant balance in many cell types, and initial signaling pathways for NF-κB invoked a direct role of ROS as a signaling intermediate (59). Furthermore, Rahman and colleagues have shown that intracellular GSH levels affect NF-κB activity (59).

Activation of cell-surface receptors leads to phosphorylation of receptor-associated kinases (11), which in turn phosphorylate the inhibitors of NF-κB kinase (IKKs). Two isoforms of IKK exist, IKK1 and IKK2, held together in a complex by the scaffolding protein IKKγ (11). IKK2 is the most important isoform for the activation of NF-κB and inflammatory gene transcription. Phosphorylation of IKKs results in phosphorylation of the NF-κB cytoplasmic inhibitor (IκBα), so that IκBα is targeted for proteosomal degradation. This degradation precipitates the release of NF-κB from its inactive state, enabling nuclear translocation and binding to specific DNA response elements within the regulatory regions of responsive genes (11).

NF-κB is predominantly composed of the p50/p65 heterodimer (5). Subtle changes in p65 phosphorylation are influential; for example, inactive p65 is nonphosphorylated and is associated predominantly with HDAC1, whereas p65 is phosphorylated following IKK-2 stimulation and is able to bind to coactivator molecules such as p300/CBP (77).

NF-κB induces histone acetylation

Cytokines such as TNF-α and IL-1β, acting via NF-κB, can induce histone acetylation in both a time- and concentration-dependent manner (30). This NF-κB–induced acetylation occurs preferentially on histone H4, rather than histones H2A, H2B, or H3, and it is directed primarily toward lysine residues 8 and 12 at NF-κB–responsive regulatory elements (30; K. Ito, unpublished observations). On DNA binding, NF-κB recruits a large coactivator complex that contains the HAT proteins CBP and PCAF, although neither of these are the major HAT activated by NF-κB (30). IL-1β can also activate other pathways, distinct from NF-κB, which can impinge on NF-κB activation (50). These additional pathways, such as protein kinase C and nonreceptor tyrosine kinases, may enhance NF-κB activity, either by phosphorylating p65 and thereby enhancing cofactor recruitment (77) or by phosphorylating NF-κB– associated cofactors (50). PM10s, acting through NF-κB, have also been reported to enhance histone acetylation in A549 cells (26).

IKK-1 is generally thought to be predominantly involved in the adaptive immune response rather than cytokine-induced NF-κB function. However, two recent articles suggest that IKK-1 can modulate NF-κB–dependent gene expression in response to TNF-α treatment (2, 76). Based on chromatin immunoprecipitation assays, it was reported that IKK-1 is recruited to the promoters of NF-κB–regulated genes in association with CBP and p65 following stimulation with TNF-α. This resulted in gene-specific phosphorylation of histone H3 on serine 10 and subsequent increased gene expression.

The HDAC inhibitor trichostatin A has been reported to enhance NF-κB-driven inflammatory gene transcription in a number of cell lines (4, 15, 30, 77). Two major mechanisms for this effect have been proposed. In the first case, it has been reported that NF-κB has an associated HDAC when bound to DNA that acts as a break on the ability of NF-κB to activate local HAT activity. Inhibition of this associated HDAC leads to increased local HAT activity and elevated inflammatory gene transcription (4, 30, 77). Warner Greene and colleagues (15) have proposed an alternative mechanism. Here, HDAC3 can modify NF-κB nuclear-cytoplasmic shuttling and association with IκBα, resulting in enhanced nuclear retention of activated NF-κB that is insensitive to inactivation by IκBα. More recently, using overexpression systems, it has been suggested that IκBα can sequester HDAC1 and HDAC3 in the cytoplasm, enhancing NF-κB activity (74). These data suggest that HDAC activity is important in the regulation of inflammatory gene expression, and further evidence for this is seen in BAL macrophages from man, where TNF-α-induced granulocyte-macrophage colony-stimulating factor (GM-CSF) release is inversely correlated with HDAC activity (32).

Thus, we are left with a model whereby ROS can activate NF-κB and several other intracellular signaling pathways such as the mitogen-activated protein (MAP) kinases, which can subsequently phosphorylate and activate transcriptional coactivators and regulators, including HATs and HDACs. This results in the induction of proinflammatory genes, which in turn can further stimulate changes in the oxidant/antioxidant balance, resulting in further inflammatory gene expression (Fig. 2).

Temporal association of NF-κB with DNA, cofactors, and gene induction

In a series of recent studies, Saccani and colleagues have shown that the simplistic model described previously needs modification (66, 67). Immediate early genes such as IκBα do

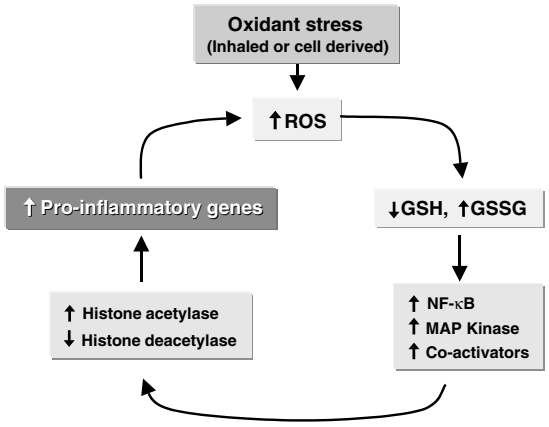

FIG. 2. Proposed model of oxidant-mediated inflammatory gene production. Induction of inflammatory genes is induced by oxidants either from cellular sources or from the environment (inhaled). This results in a depletion/oxidation of the antioxidant GSH and an alteration in the cellular redox balance. This change in redox state signals for changes in the activation of intracellular signaling pathways such as MAP kinases and other coactivators and subsequent enhancement of nuclear acetylase activity and decreased deacetylase activity. This finally leads to enhanced transcription of inflammatory mediators and a feed-forward further induction of MAP kinase and NF-κB activation, changes in histone acetylation, and increased inflammatory gene expression.

indeed display binding of NF-κB to their promoters rapidly after lipopolysaccharide (LPS) stimulation, but within 10 min NF-κB dissociates from the IκBα promoter site and never reassociates. In contrast, NF-κB binds to its promoter sites in DNA for up to 2 h before dissociation in distinct sets of genes (MnSOD and MIP-2), in spite of stimulation by LPS at the same time. However, other NF-κB–regulated genes, such as RANTES, monocyte chemoattractant protein-1, and IL-6, do not show NF-κB binding to their promoters until 2 h after activation. NF-κB sites in the promoter regions of these genes are originally in a repressed chromatin environment that prevents NF-κB DNA binding and subsequent gene expression. These become accessible only after AP-1–mediated histone acetylation and subsequent alteration in the local nucleosomal structure (66, 67).

GLUCOCORTICOID-INDUCED GENE TRANSCRIPTION

Glucocorticoids exert their effects by binding to a cytoplasmic receptor (GR) (38). Inactive GR is bound to a protein complex that includes two subunits of the heat shock protein hsp90, which act as molecular chaperones, preventing the nuclear localization of the unoccupied GR (38). Once the ligand binds to GR, hsp90 dissociates, allowing nuclear localization of the activated GR–steroid complex, binding as a dimer to glucocorticoid response elements (GREs), and GR interaction with coactivator complexes (38).

GR, like other transcription factors, increases gene transcription through an action on chromatin remodeling and recruitment of RNA polymerase II to the site of local DNA unwinding, as described above for NF-κB. GR interacts with CBP and other coactivator proteins, including steroid receptor coactivator-1 (SRC-1), which enhance local HAT activity (30, 63). Dexamethasone at high concentrations ($\geq 10^{-8}\,M$) in A549 cells enhances binding of activated GR to CBP and/ or associated coactivators, resulting in histone acetylation on lysines 5 and 16 of histone H4 and increased gene transcription (30). Furthermore, recent data from O'Malley and colleagues show that differential recruitment of coactivators by nuclear receptors determines the assembly of coactivator complexes on target promoters, resulting in acetylation of distinct lysine residues at the mouse mammary tumor virus (MMTV) promoter (46).

Histone H1 phosphorylation may also play a role in gene expression activated by the GR (44). Only the phosphorylated form of histone H1 can be displaced from the MMTV promoter by the GR. Furthermore, long exposure to glucocorticoids leads to H1 dephosphorylation (44).

The question arises: how can the GR, or any other transcription factor, interact with its recognition site when DNA is compacted? GR may bind to a GRE within the linker DNA between nucleosomes or, alternatively, when the GRE is wound around histones, as long as the core residues are facing outward (45). Binding to the GRE may then modify the local chromatin structure, altering GR access. High-resolution mapping of GR interactions with the MMTV-long terminal repeat in *Xenopus* oocytes suggests that the GR not only reorganizes the chromatin immediately surrounding its binding site, but also can have effects elsewhere, thereby enforcing a particular translational frame on the chromatin template and modifying the effects of other DNA binding proteins (12).

The number of genes directly regulated by glucocorticoids is estimated to be 10–100, but many genes are indirectly regulated through an interaction with other transcription factors and coactivators (10). It is unlikely, however, that the widespread antiinflammatory actions of glucocorticoids could be explained by increased transcription of a small number of antiinflammatory genes, such as annexin-1, IL-10, and IκBα. The induction of these genes, and others, is cell-specific, with IκBα induction being more important in T-cells than epithelial cells (29, 68).

Cross-talk between the GR and other transcription factors

The major antiinflammatory effects of glucocorticoids are through repression of inflammatory and immune genes due largely to interaction between the activated GR and the transcription factors, such as NF-κB and AP-1, that mediate the expression of these inflammatory genes (38).

An important question is why glucocorticoids only switch off inflammatory genes, as they clearly do not suppress all activated genes and are well tolerated as long-term treatments. It is possible that the GR, acting as a monomer, binds only to specific coactivator complexes that are activated by proinflammatory transcription factors, such as NF-κB and AP-1, although we do not understand how this specific recognition occurs.

The interaction between proinflammatory transcription factors and the GR may result in differing effects on histone

acetylation/deacetylation, through one of several mechanisms that are probably not exclusive. The repressive action of glucocorticoids may be due to competition-activated GR binding to one of several transcription corepressor molecules, such as NCoR-1, which associate with proteins that have differing HDAC activity (63). In addition, IL-1β and TNF-α can cause histone acetylation on lysines 8 and 12 of histone H4, and low concentrations of dexamethasone (>10^{-9} *M*) can repress this IL-1β–stimulated histone acetylation. This occurs by direct inhibition of CBP-associated HAT activity and by active recruitment of HDAC proteins (30) (Fig. 3). In addition, high concentrations of glucocorticoids can induce HDAC expression in a time-dependent manner (30). Overall, this process results in the deacetylation of histones and repression of inflammatory genes (30).

According to the "histone code" (36), other histone modifications would be expected to play a role in GR/NF-κB cross-talk at the level of chromatin. Recent evidence has indicated that the off-switch for NF-κB–mediated inflammatory gene transcription correlates with histone H3 K9 methylation rather than decreased H4 acetylation (65). We have recently shown that suppression of histone methylation blocks GR function, synergistically with inhibition of HDACs (37). Previous reports of competition between pro- and antiinflammatory transcription factors for limited amounts of cofactors, such as CBP, playing a role in GR-transcription factor cross-talk are unlikely to be important (19).

The importance of cross-talk in GR actions is indicated by the construction of a GR dimerization-deficient mutant mouse in which the GR is unable to dimerize and therefore bind to DNA, so that the transactivation and transrepression activities of glucocorticoids are separated (60, 61). These animals survive to adulthood, in contrast to GR-knockout animals. In these animals, dexamethasone was able to inhibit AP-1–driven and NF-κB–driven gene transcription, but the ability to facilitate GRE-mediated effects, such as cortisol suppression and T-cell apoptosis, was markedly attenuated. It is proposed that the detrimental side effects of glucocorticoids, which limit their use in ever increasing doses, are due predominantly to GRE-mediated events, whereas the antiinflammatory properties are mediated via transrepression, these data suggest that it will be possible to develop glucocorticoids (dissociated steroids) with a greater therapeutic window. Whether these assumptions are valid is likely to be revealed when these compounds are used therapeutically in man.

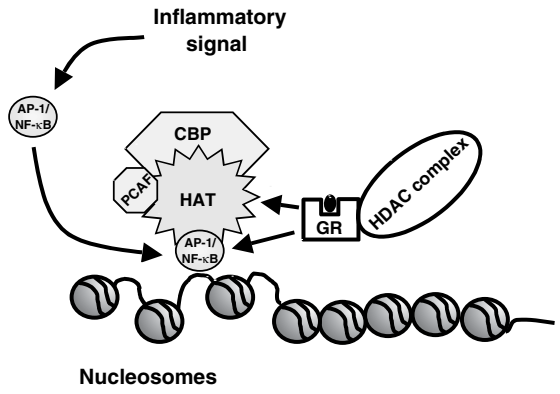

FIG. 3. How glucocorticoids switch off inflammatory genes. Inflammatory genes are activated by inflammatory stimuli, such as IL-1β or TNF-α, resulting in activation of the transcription factor NF-κB. A dimer of p50 and p65 NF-κB proteins translocates to the nucleus and binds to specific κB recognition sites and also to coactivators, such as CBP or PCAF, which have intrinsic HAT activity. This results in acetylation of lysines in core histone-4, resulting in increased expression of genes encoding inflammatory proteins, such as GM-CSF. GRs after activation by corticosteroids translocate to the nucleus and bind to coactivators to inhibit HAT activity directly. In addition, GR is able to recruit HDACs, which reverse histone acetylation, to the NF-κB complex leading to suppression of inflammatory genes.

EFFECTS OF OXIDATIVE STRESS ON GR FUNCTION

An important characteristic of the inflammation in COPD is the lack of response to glucocorticoids, which has been linked to oxidative stress (16, 17). Cigarette smoking is the primary cause of COPD, and the smoke contains >10^{15} oxidant molecules per puff (62). This suggests that oxidative stress may be an important factor in inducing glucocorticoid resistance in COPD. The resistance may be due to cigarette smoking itself, because glucocorticoids are much less effective in reducing inflammatory cells in bronchoalveolar lavage fluid and sputum from smoking asthmatic patients compared with nonsmoking patients (14).

ROS may impinge on several steps in the GR activation pathway. For example, in COS7 and CHO cells, oxidative stress (100 μ*M* H_2O_2) reduced GR nuclear transport (52), and similar results were seen in pituitary cells (3). Nitrosyl stress induced by the NO donor *S*-nitroso-DL-penicillamine has also been shown to prevent GR dissociation from the hsp90 complex and a reduction in ligand binding (25).

Other potential causes of reduced GR function involve nuclear events. Proinflammatory cytokines, such as IL-1β and TNF-α, can activate the redox-sensitive transcription factors AP-1 and NF-κB, whose activities can be further enhanced in the presence of ROS and RNS (55). ROS can also activate MAP kinase pathways, particularly c-Jun N-terminal kinase and p38, and phosphatidylinositol 3-kinase (55). As a result, further transcription factors such as ATF-2, Elk-1, and coactivators such as CBP are stimulated, leading to enhanced inflammatory gene expression. Enhanced activation of some of these factors, particularly AP-1 and p38 MAP kinase, has have been postulated to account for attenuation of GR function in steroid-resistant asthmatics (1).

We have recently shown that H_2O_2 induced a time-dependent increase in histone acetylation that was maintained for 12 h. This was associated with increased IL-8 production. HAT activity was found to be highest in G2/M and equivalent in G0/G1 and S phases of the cell cycle (70). This induction of HAT activity by H_2O_2 is associated with NF-κB and AP-1 activation (56, 58, 70) and presumably binding of these factors to the IL-8 promoter (42). Similar results have been reported for activation of histone acetylation by PM10s (26). Oxidative stress can also lead to acetylation of transcription factors,

such as Sp1, and thereby enhancing transcriptional activity (64). Enhanced localized HAT activity may subsequently be less amenable to suppression by GR-recruited HDACs.

ROS stimulation of cells can also lead to increased histone H3 phosphorylation via an extracellular signal-regulated kinase-mediated process resulting in cell death (69). This effect can be attenuated by inhibitors of poly(ADP) ribosylation (PARP-1), suggesting that these events are linked. Similar results have been reported linking H_2O_2-induced histone H4 acetylation to poly(ADP) ribosylation (13) and possibly involving NF-κB and p300 (27). Increased histone H1 phosphorylation and histone H4 acetylation are again likely to result in an enhanced inflammatory response and induction of a relative glucocorticoid insensitivity.

As HDAC2 is important in GR function, we have investigated HDAC2 expression and activity in the presence of oxidative stress. HAT activity is slightly increased and HDAC2 activity is greatly decreased in BAL macrophages and biopsies of smokers and patients with COPD, and this correlates with increased inflammatory gene expression and reduced steroid responsiveness to LPS-induced IL-8 and TNF-α production (32). This effect can also be mimicked *in vitro* and *ex vivo* in epithelial cells and macrophages with H_2O_2 or the NO donor SIN-1 and parallels the effects seen with the HDAC inhibitor trichostatin A (32). Furthermore, the antioxidant *N*-acetylcysteine was able to prevent the enhanced cytokine release and partially restore dexamethasone sensitivity to these cells. This suggests that oxidative stress by repression of HDAC activity can modulate GR function in BAL macrophages and U937 cells.

This effect is specific to certain HDACs because there is a specific reduction in HDAC2 and HDAC5 expression and activity in peripheral lung and BAL macrophages from smokers and COPD patients compared with nonsmokers (31, 32). Furthermore, the loss in enzymic activity is associated with tyrosine nitration of HDAC2 (31). Tyrosine nitration is also inversely correlated with HDAC2 activity in immunoprecipitated HDAC2 isolated from the peripheral lung of aged-matched healthy smokers and COPD patients (31). Tyrosine nitration overcomes the effects of phosphorylation on distinct residues and suggests a novel mechanism whereby oxidative stress may reduce steroid responsiveness in severe airway disease. The mechanism for this action is not known, but may involve steric hindrance or nitration of a tyrosine residue (Y146) within the active site of HDAC2. HDAC phosphorylation can either enhance or decrease HDAC enzymic activity depending on the target HDAC (43). Increased HDAC2 phosphorylation on serine, threonine, or tyrosine residues following an inflammatory insult results in increased HDAC activity that may be associated with resolution of ongoing inflammatory gene transcription. Therefore, tyrosine nitration can reduce HDAC2 activity and thereby enhance inflammatory gene transcription. Furthermore, this will result in reduced steroid sensitivity, as HDAC2 recruited to the NF-κB activation complex will also have reduced activity (Fig. 4). This suggests that induction of HDAC activity would be useful in COPD.

In contrast, HDAC inhibitors have been developed for the treatment of several oncogenic diseases due to their ability to induce cell-cycle arrest and prevent inflammatory gene expression (34, 40). The ability to prevent cell proliferation is due to induction of $p21^{CIP}$, an inhibitor of cyclin-dependent kinase 2 and cell-cycle progression, and is associated with inhibition of specific HDAC isoforms (40). However, there is some dissociation between HDAC inhibition and regulation of cell proliferation (18, 24). For example, trichostatin A inhibits HDAC activity (IC_{50} = 5 n*M*) at much lower concentrations than that required to inhibit proliferation in A549 cells (IC_{50} = 600 n*M*), which suggests that these inhibitors may be acting through alternative mechanisms at high concentrations. Furthermore, it is becoming clear that different HDACs perform distinct roles in the regulation of proliferation and inflammatory genes (20, 49). It may be possible in the future to obtain selective inhibitors or activators of individual HDAC isoforms that control inflammatory gene expression.

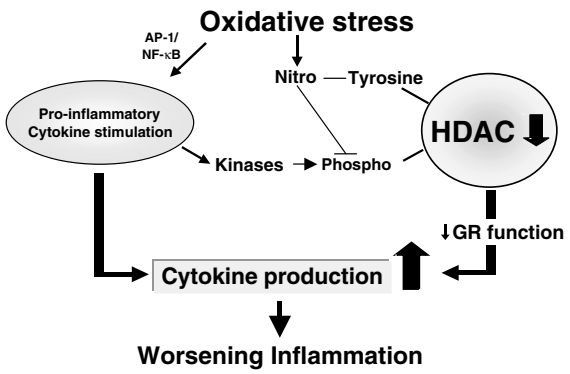

FIG. 4. Proinflammatory cytokine production is induced by inflammatory stimuli following activation of kinase cascades. These same cascades act in a negative feedback manner to switch off cytokine mRNA production after an initial burst of production. This is due, at least in part, to phosphorylation of HDACs, increasing their activity and thus inducing a silenced chromatin structure. Oxidative stress overcomes this feedback activity of HDAC activity by inducing nitration of distinct tyrosine residues and repressing HDAC activity. Similar effects may occur on GR itself to modify its actions in response to oxidative stress.

FUTURE PROSPECTS

The elucidation of the molecular mechanisms of glucocorticoids raises the possibility that novel nonsteroidal antiinflammatory treatments might be developed that mimic the actions of glucocorticoids on inflammatory gene regulation. Inhibition of specific HATs activated by NF-κB may prove to be useful targets, especially if they also repress the action of other proinflammatory transcription factors (72). Alternatively, activation of HDACs may have therapeutic potential, and theophylline has been shown to have this property, resulting in marked potentiation of the antiinflammatory effects of glucocorticoids both *in vitro* and *in vivo* (33). This action of theophylline is not mediated via phosphodiesterase inhibition or adenosine receptor antagonism and, therefore, appears to be a novel action of theophylline (8). HDAC phosphorylation also affects their activity (30, 71), and changes in HDAC2 phosphorylation may be the mechanism of theophylline ac-

tion (33). It may be possible, therefore, to discover similar drugs that could form the basis of a new class of antiinflammatory drugs without the side effects that limit the use of theophylline (8) or to find inhibitors of specific kinases or phosphatases that result in up-regulation of HDAC activity.

CONCLUSION

Advances in delineating the fundamental mechanisms of gene transcription, especially recruitment of histone-modifying cofactors, have resulted in better understanding of the molecular mechanisms whereby glucocorticoids and oxidative stress modulate inflammation. The challenge is to see if these mechanisms hold true in primary cells *in vivo*. This will undoubtedly lead to the development of drugs that target novel aspects of GR function and potentially restore glucocorticoid sensitivity to diseases that are unresponsive to current therapeutic strategies.

ACKNOWLEDGMENTS

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ABBREVIATIONS

AP-1, activator protein-1; BAL, bronchoalveolar lavage; CBP, CREB binding protein; COPD, chronic obstructive pulmonary disease; GM-CSF, granulocyte-macrophage colony-stimulating factor; GR, glucocorticoid receptor; GRE, glucocorticoid response element; GSH, glutathione; HAT, histone acetyltransferase; HDAC, histone deacetylase; H_2O_2, hydrogen peroxide; hsp90, heat shock protein-90; IKK, inhibitor κB kinase; IL, interleukin; LPS, lipopolysaccharide; MAP, mitogen-activated protein; MIP, macrophage inflammatory protein; MMTV, mouse mammary tumor virus; NF-κB, nuclear factor-κB; NO, nitric oxide; $O_2^{\bullet -}$, superoxide anion; $^{\bullet}OH$, hydroxyl radical; PCAF, p300/CBP-associated factor; PM10, particulate matter ≤10 μm in diameter; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; SRC-1, steroid receptor coactivator-1; TNF-α, tumor necrosis factor-α.

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Address reprint requests to:
Ian M. Adcock, Ph.D.
Thoracic Medicine
National Heart and Lung Institute
Imperial College London
Dovehouse Street
London, SW3 6LY, U.K.

E-mail: ian.adcock@imperial.ac.uk

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