Am J Physiol Lung Cell Mol Physiol 291: L46–L57, 2006.
First published February 10, 2006; doi:10.1152/ajplung.00241.2005.

Cigarette smoke induces proinflammatory cytokine release by activation of NF-κB and posttranslational modifications of histone deacetylase in macrophages

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Submitted 3 June 2005; accepted in final form 16 January 2006

Yang, Se-Ran, Asiya S. Chida, Mark R. Bauter, Nusrat Shafiq, Kathryn Seweryniak, Sanjay B. Maggirwar, Iain Kilty, and Irfan Rahman. Cigarette smoke induces proinflammatory cytokine release by activation of NF-κB and posttranslational modifications of histone deacetylase in macrophages. *Am J Physiol Lung Cell Mol Physiol* 291: L46–L57, 2006. First published February 10, 2006; doi:10.1152/ajplung.00241.2005.—Cigarette smoke-mediated oxidative stress induces an inflammatory response in the lungs by stimulating the release of proinflammatory cytokines. Chromatin remodeling due to histone acetylation and deacetylation is known to play an important role in transcriptional regulation of proinflammatory genes. The aim of this study was to investigate the molecular mechanism(s) of inflammatory responses caused by cigarette smoke extract (CSE) in the human macrophage-like cell line MonoMac6 and whether the treatment of these cells with the antioxidant glutathione (GSH) monoethyl ester, or modulation of the thioredoxin redox system, can attenuate cigarette smoke-mediated IL-8 release. Exposure of MonoMac6 cells to CSE (1% and 2.5%) increased IL-8 and TNF-α production vs. control at 24 h and was associated with significant depletion of GSH levels associated with increased reactive oxygen species release in addition to activation of NF-κB. Inhibition of IKK ablated the CSE-mediated IL-8 release, suggesting that this process is dependent on the NF-κB pathway. CSE also reduced histone deacetylase (HDAC) activity and HDAC1, HDAC2, and HDAC3 protein levels. This was associated with posttranslational modification of HDAC1, HDAC2, and HDAC3 protein by nitrotyrosine and aldehyde-adduct formation. Pretreatment of cells with GSH monoethyl ester, but not thioredoxin/thioredoxin reductase, reversed cigarette smoke-induced reduction in HDAC levels and significantly inhibited IL-8 release. Thus cigarette smoke-induced release of IL-8 is associated with activation of NF-κB via IKK and reduction in HDAC levels/activity in macrophages. Moreover, cigarette smoke-mediated proinflammatory events are regulated by the redox status of the cells.

oxidants; glutathione; MonoMac6 cells; IκB kinase; chronic obstructive pulmonary disease

CIGARETTE SMOKING IS THE MAJOR etiologic factor in the pathogenesis of chronic obstructive pulmonary disease (COPD) (41), which is characterized by an abnormal inflammatory response in the lungs to cigarette smoke with a progressive and irreversible airflow limitation. Chronic airway inflammation is an archetypal feature of COPD, and increased oxidative stress has been suggested to be responsible for triggering inflammatory events observed within the lungs of smokers and COPD patients. Although the precise mechanisms behind the pathogenesis of COPD are yet to be fully dissected, the current hypothesis suggests that cigarette smoke causes airway inflammation by activating macrophages, neutrophils, and T lymphocytes, which release proteases and reactive oxygen species (ROS) leading to cellular injury (14, 48). As a consequence, chronic inflammatory processes are triggered that lead to small airway obstruction. An increased oxidant burden in smokers may be derived from the fact that cigarette smoke contains an estimated 10^{17} oxidants/free radicals and 4,700 chemical compounds, including reactive aldehydes (carbonyls) and quinones, per puff. Many of these are relatively long-lived, such as tar-semiquinone, which can generate hydroxyl radicals (·OH) and H_2O_2 by the Fenton reaction (36). One consequence of this increased oxidative stress is activation of redox-sensitive transcription factors, such as NF-κB and activator protein-1 (AP-1), which are critical to transcription of proinflammatory genes (IL-8, IL-6, and TNF-α) (37, 43). However, the precise transcriptional mechanisms leading to enhanced gene expression in response to cigarette smoke are still not clearly understood.

Macrophages are derived from monocytes and are suggested to be the main orchestrators of the chronic inflammatory response and tissue destruction observed in patients with COPD (2, 50, 51). For example, the number of alveolar macrophages has been found to increase in proportion with disease severity in COPD patients. Furthermore, macrophages from these patients release increased levels of proinflammatory cytokines and matrix metalloproteinases compared with macrophages from nonsmoking control subjects (2, 29). This suggests that these cells play an important role in perpetuation of inflammatory responses in the pathogenesis of COPD.

IL-8 is a multifunctional cytokine that has significant neutrophil chemoattractant and activating properties. It is produced by a variety of inflammatory and pulmonary cell types in response to oxidative stress, a process thought to be mediated by upregulation of redox-sensitive transcription factors such as NF-κB and AP-1. Although oxidative stress induces IL-8 release from lung alveolar epithelial cells, the molecular mechanisms of cigarette smoke-mediated cytokine production in macrophages have not been investigated. To study the effects

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of cigarette smoke exposure at the cellular level in vitro, we have investigated the utility of a monocytic-macrophage cell line, MonoMac6, to better understand the proinflammatory effects of cigarette smoke.

Histone deacetylation by histone deacetylases (HDACs) removes acetyl moieties from lysine residues of histones, causing rewinding of DNA and hence silencing gene transcription. The HDAC family of enzymes has been reported to have 17 isoforms, each differentially expressed and regulated in different cell types (8). HDACs not only cause the inhibition of gene transcription but also directly affect the nuclear binding of transcription factors such as NF-κB. It was recently reported that HDACs 1 and 2 play a key role in the regulation of cell proliferation and corticosteroid-mediated inhibition of proinflammatory mediator expression (17, 49). Corticosteroids recruit HDAC2 protein to the NF-κB transcriptional complex, causing chromatin condensation and suppressing proinflammatory gene transcription. Any alteration or modification of HDAC2 by oxidative stress/cigarette smoke may therefore render corticosteroids ineffective, as reported in patients with COPD (43, 44). It was also shown recently that HDAC1 and HDAC3 can interact directly with the p65 subunit of NF-κB to exert a corepressor function in the nucleus (4, 53). Therefore, NF-κB-mediated transcription is regulated by HDAC1, HDAC2, and HDAC3. However, oxidant-mediated regulation of these enzymes has not been described.

Because cigarette smoke is also known to induce oxidative stress, the effects of cigarette smoke on the regulation of HDAC1, HDAC2, and HDAC3 expression and HDAC enzyme activity were investigated. We hypothesize that cigarette smoke extract (CSE) inhibits deacetylation and activates NF-κB, leading to release of proinflammatory cytokines in monocyte-derived macrophages. Therefore, we investigated the effect of cigarette smoke on HDAC1, HDAC2, and HDAC3 levels, HDAC activity, transactivation of NF-κB, interaction of HDAC protein with NF-κB p65/RelA, and the release of IL-8 from MonoMac6 cells. We also investigated whether elevation of intracellular glutathione (GSH) by GSH monoethyl ester (GSHMEE) and thioredoxin/thioredoxin reductase (TR/TRX) can prevent the cigarette smoke-mediated reduction in HDAC activity and IL-8 release.

MATERIALS AND METHODS

Unless otherwise stated, all biochemical reagents used in this study were purchased from Sigma (St. Louis, MO).

MonoMac6 Cell Culture

The human monocytic cell line (mature monocytes) MonoMac6, which was established from peripheral blood of a patient with monoblastic leukemia (28, 54), was grown in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum (HyClone Laboratories, Logan, UT), 2 mM L-glutamine, 100 μg/ml penicillin, 100 U/ml streptomycin, 1% nonessential amino acids, 1% sodium pyruvate, 1 μg/ml human holo-transferrin, and 1 mM oxaloacetic acid. The cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2.

Preparation of Aqueous CSE

Research-grade cigarettes (1R3F) with a filter from the Kentucky Tobacco Research and Development Center at the University of Kentucky (Lexington, KY) were smoked to 0.5 cm above the filter in a fume hood, using a modification of the method developed by Carp and Janoff (3) and as previously described (27, 30). Ten percent CSE was prepared by bubbling smoke from 2 cigarettes into 20 ml of serum-free RPMI at a rate of 1 cigarette/min. The pH of the RPMI was adjusted to 7.4, the optical density was determined (0.81 ± 0.03 at 350 nm), and the medium was sterile filtered with a 0.45-μm filter (25-mm Acrodisc; Pall, Ann Arbor, MI). The CSE was always prepared fresh on the day of the experiment. The pattern of absorbance (spectrogram) at 320 nm observed showed very little difference between different preparations of CSE. We prepared control medium by bubbling air through 20 ml of serum-free RPMI, adjusting pH to 7.4, and sterile filtering as described above.

Treatments

MonoMac6 cells were seeded at a density of 1.5×10^6 cells in six-well culture plates in a total final volume of 2 ml, grown to ~80–90% confluence in six-well plates containing RPMI with 10% FBS, washed in Ca^{2+}/Mg^{2+}-free PBS, and exposed to the treatments in medium containing 1% serum. All treatments were performed in duplicate. The cells were treated with either H_2O_2 (100 μM) or CSE (1, 2.5, 5%), IKK inhibitor (IKKi), an analog of UK-436303 (2 μM; Pfizer), NF-κB inhibitor BAY-117082 (20 μM; Biomol, Plymouth Meeting, PA), trichostatin A (TSA; 100 ng/ml), bacterial LPS (*Escherichia coli*) (1 μg/ml), and TNF-α (10 ng/ml) for 4 and 24 h at 37°C with 5% CO_2. The cells were washed with cold sterile Ca^{2+}/Mg^{2+}-free PBS and lysed before being used to assay HDAC1, HDAC2, and HDAC3 levels and HDAC activity. Culture media from these macrophages were collected and stored at −80°C until analyzed. The culture media were used for IL-8 and TNF-α assay.

To investigate the effect of the thiol compound GSH and the TR/TRX redox system on CSE-mediated regulation of HDAC and IL-8 release, cells were pretreated with 1 mM GSHMEE for 2 h, followed by washing and treatment with CSE at concentrations of 1%, 2.5% and 5% for 24 h. Similarly, cells were also pretreated with *E. coli* thioredoxin (1 μM), *E. coli* thioredoxin reductase (0.1 μM), and reduced NADP (NADPH; 2 μM).

Assessment of Cellular GSH Concentration

Intracellular GSH levels were determined according to the method of Tietze (52) with slight modifications (45). Briefly, the cells were harvested, washed in Ca^{2+}/Mg^{2+}-free PBS, and then sonicated in 0.1 M phosphate buffer containing 5 mM EDTA, 0.1% (vol/vol) Triton X-100, and 0.6% (wt/vol) sulfosalicylic acid. The cellular debris was collected by centrifugation, the supernatant was incubated with 0.2 mg/ml DTNB and 1.67 U/ml glutathione reductase in phosphate buffer-EDTA for 30 s, 0.2 mg/ml β-NADPH was added, and the rate of DTNB reduction was spectrophotometrically measured at 405 nm. The concentration of GSH in the supernatant was determined by comparison with the rate of DTNB reduction by known concentrations of GSH. For the glutathione disulfide (GSSG) assay, the supernatant was treated with 2-vinylpyridine and triethanolamine as described previously (13) and thereafter was used in the assay for GSH as described above.

Measurement of ROS by Flow Cytometry

Oxidative stress in terms of ROS was measured by flow cytometry using 5-(-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) dye. The cells were seeded at a density of 2.5×10^5 cells in six-well culture plates in a total final volume of 2 ml and grown to ~80–90% confluence in six-well plates containing RPMI with 1% FBS. Freshly prepared CSE was added, and cells were incubated for a period of 4, 12, and 24 h. The cells were harvested, washed with cold sterile PBS, resuspended in 1 ml of PBS, and incubated with or without H_2DCFDA (10 μM) for 0.5 h. Cells were washed and resuspended in PBS. Flow cytometric analysis was performed with an

Elite flow cytometer (Coulter). Dead cells, detected by propidium iodide (10 μg/ml), as well as forward and side scatter, were gated out. A minimum of 10,000 events were acquired for each sample. Debris was gated out, and analysis was performed only on the live monocytic population.

HDAC Activity

HDAC activity was measured with a colorimetric assay kit (Biomol). The procedure involves the use of the HDAC colorimetric substrate (Color de Lys substrate, 500 μM), which comprises an acetylated lysine side chain and is incubated with a sample containing nuclear extract. Deacetylation sensitizes the substrate, and treatment with the lysine developer produces a chromophore, which can be analyzed with a colorimetric plate reader at 405 nm. HeLa cell nuclear extract was used as a positive control. A standard curve was prepared, using the known amount of the deacetylated standard (Boc-Lys-pNA) included in the kit.

Western Blotting for HDAC Proteins

Cellular nuclear proteins were extracted from treated MonoMac6 cells with 10% Nonidet P-40 lysis buffer supplemented with a protease inhibitor cocktail (leupeptin, aprotinin, pepstatin, and PMSF). Protein quantitation was done by the bicinchoninic (BCA) acid method, following the manufacturer's instructions (Pierce, Rockford, IL). For HDAC1, HDAC2, and HDAC3 assays, 20 μg of isolated soluble proteins was electrophoresed on 7.5% PAGE gels, electroblotted onto nitrocellulose membranes (Amersham), and blocked with 10% nonfat dry milk in Tris-buffered saline (TBS) with 0.1% Tween 20 at 4°C overnight. Membranes were incubated with goat polyclonal anti-human anti-HDAC (HDAC1, -2, -3; 1:1,000 dilution in 5% nonfat dry milk in TBS) antibodies (HDAC1 SC-6298, HDAC2 SC-6296, HDAC3 SC-8138; Santa Cruz) (25, 30, 53). After washing, the levels of HDAC protein were detected with rabbit anti-goat antibody (1:20,000 dilution in 2.5% nonfat dry milk in TBS for 1 h) linked to horseradish peroxidase (Dako), and bound complexes were detected with enhanced chemiluminescence (Jackson Lab).

HDAC Immunoprecipitation and Posttranslational Modification of HDACs

MonoMac6 cells were treated with CSE (1%, 2.5%, and 5%) for 24 h at 37°C, the nuclear fraction was isolated, and HDAC1, HDAC2, and HDAC3 were immunoprecipitated. HDAC1, -2, and -3 antibody (Santa Cruz; 1:1,000 dilution) was added to 200 μg of protein in a final volume of 100 μl and incubated for 1 h. Protein A/G agarose beads (40 μl; Santa Cruz) were added to each sample and left for 4 h at 4°C on a rocker. The samples were then centrifuged at 13,000 rpm at 4°C for 5 min. The supernatant was discarded, and the beads were washed twice and then resuspended in 150 μl of lysis buffer. The assay was allowed to continue at 37°C for 90 min with continuous mixing. After this time, the agarose beads were pelleted by centrifugation, the supernatant was transferred to a 96-well plate, and the amount of HDAC1, HDAC2, and HDAC3 product formed was measured spectrophotometrically at 405 nm. For Western blots, 20 μg of the immunoprecipitated HDAC1, HDAC2, and HDAC3 agarose bead suspension was added to 20 μl of 5× sample buffer, boiled, and resolved by SDS-PAGE as described above. Negative alsone (beads only) and IgG R were used as negative controls and cell lysate from NIH 3T3 cells as positive control. To demonstrate the interaction of HDAC2 protein with p65 subunit of NF-κB, 20 μg of immunoprecipated HDAC2 was blotted against p65/RelA. To determine the posttranslational modification of HDAC1, -2, and -3, blots were reprobed with mouse monoclonal anti-nitrotyrosine antibody (Upstate catalog no. 05–223), stripped, and reprobed with mouse monoclonal anti-4-hydroxy-2-nonenal (4-HNE) antibody (Oxis International catalog no. 24327) and NF-κB p65/RelA (Santa Cruz).

Transient Transfection of NF-κB-Luciferase Reporter Gene in MonoMac6 Cells

MonoMac6 cells were transfected (2 μg DNA/well; 10-to-1 ratio) with NF-κB-response luciferase plasmid pGL3pro or pGL3–3kBpro (a kind gift from Dr. Takashi Nagaya, Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan) (24), using a Lipofectamine 2000 reagent (Invitrogen) in serum-free medium, and then incubated (15 min, 37°C) before adding to cells. After a 24-h incubation of the cells with liposome-DNA solution, 2× fresh medium was added to the solution. After an additional 24-h incubation, various treatments were carried out. The cells were lysed in passive lysis buffer (Promega, Madison, WI), and the luciferase activity in the cell lysates was determined by a luminometer and normalized with protein contents of the cell lysates. Experiments were repeated, using at least three separate times.

Electrophoretic Mobility Shift Assay

Nuclear protein was extracted from the cells as described previously (30, 39). Labeling reaction was performed with NF-κB-specific double-stranded oligonucleotide (Promega) with [γ-^{32}P]ATP. Binding experiments were performed with 5 μg of nuclear protein, 2 μl of 5× binding buffer [50 mM HEPES, pH 7.5, 500 mM NaCl, 25% glycerol, 5 mM EDTA, 0.25 mg/ml poly(dI-dC)·poly(dI-dC)], and 1 μl of the radiolabeled NF-κB-specific oligonucleotide 5′-AGT TGA GGG GAC TTT CCC AGG C-3′, 3′-TCA ACT CCC CTG AAA GGG TCC G-5′ (Promega) for 20 min at room temperature. Nondenaturing 8% polyacrylamide gel electrophoresis was performed with 0.5× Tris borate-EDTA buffer, pH 8.0 for 4 h at 100 V. The gel was dried for 30 min, after which the autoradiography was carried out. To monitor the specificity of the binding reaction, the assay was performed in the presence of 1,000-fold excess of the nonlabeled oligonucleotide (30, 39), negative water control, positive HeLa nuclear extract control, and cold competitor using 100-fold molar excess of unlabeled oligonucleotides.

ELISA for IL-8 and TNF-α

The culture medium was collected after treatment and centrifuged at 2,500 rpm for 5 min to pellet the cells. The supernatant was then removed and stored at −20°C before analysis. The levels of IL-8 and TNF-α in the supernatant were determined by sandwich ELISA, using the respective dual antibody kits (R&D Systems) according to the manufacturer's instructions.

Protein Assay

Protein levels were measured with a BCA kit (Pierce). Protein standards were obtained by diluting a stock solution of BSA. Linear regression was used to determine the actual protein concentration of the samples.

Statistical Analysis

Results are shown as means ± SE of three experiments. Statistical analysis of significance was calculated with one-way ANOVA followed by Tukey's post hoc test for multigroup comparisons, using STATVIEW and Sigma plot statistical packages.

RESULTS

CSE Induces IL-8 and TNF-α Release by Depletion of Intracellular GSH Levels in MonoMac6 Cells

To determine the effect of CSE on IL-8 release, we treated MonoMac6 cells with CSE (1%, 2.5%, and 5%) for 24 h. CSE treatment (1% and 2.5%) significantly increased IL-8 release from these cells at 24 h of treatment ($P < 0.001$; Fig. 1).

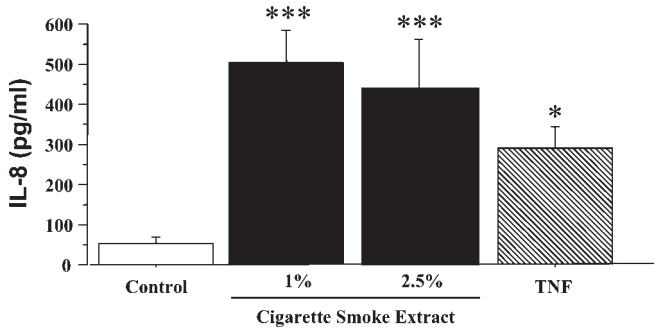

Fig. 1. Cigarette smoke extract (CSE) induces IL-8 release from MonoMac6 cells. MonoMac6 cells were treated with freshly prepared CSE (1%, 2.5%, and 5%) and TNF-α as a positive control (10 ng/ml) for 24 h, cells were harvested, and supernatants were collected for measuring IL-8 levels by sandwich ELISA. CSE at 1% and 2.5% showed increased levels of IL-8, whereas 5% CSE did not show any significant change in IL-8 release. Each histogram represents the mean of 3–4 experiments. Statistically significant differences compared with control values. $*P < 0.05$, $***P < 0.001$.

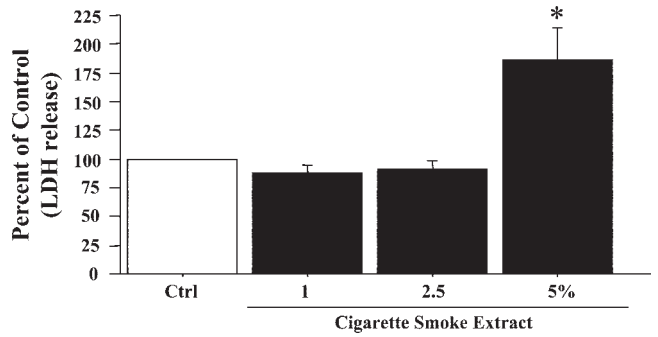

Fig. 2. CSE-induced cytotoxicity measured as lactate dehydrogenase (LDH) release in MonoMac6 cells. Cells were exposed to increased concentrations of CSE (1%, 2.5%, and 5%) for 24 h. After the incubation period, cells were harvested and supernatant was used to determine the cytotoxicity at 410 nm by LDH assay with a Roche kit. Five percent CSE exposure showed significant cell toxicity compared with low doses of CSE. One hundred percent control lysis was prepared by adding 1% Triton X-100 to the control cells. $*P < 0.05$ compared with control values (n = 8–10).

Interestingly, CSE at a higher concentration (5%) did not increase IL-8 release (pg/ml: control 52 ± 7.5 vs. 5% CSE 46 ± 3.2; $n = 4$, $P > 0.05$). However, by normalizing the percentage of live cells vs. dead cells and relating that to IL-8 release, we found that CSE (1%, 2.5%, and 5%) dose-dependently increased IL-8 release from these cells (IL-8 release-to-live cells ratio: control 57%, 1% CSE 349%, 2.5% CSE 444%, and 5% CSE 684%). Similarly, a significant increase in IL-8 release was observed with 1% and 2.5% CSE (pg/ml: 1% CSE 376 ± 21, 2.5% CSE 742 ± 51 pg/ vs. controls 99 ± 29; $n = 3$, $P < 0.01$), but not by 5% CSE (54 ± 18 pg/ml; $P > 0.05$), in another monocytic cell line (histiolytic monocytes, U937). In addition, CSE treatment (1% and 2.5%) significantly increased TNF-α release from MonoMac6 cells at 24 h of treatment (pg/ml: 1% CSE 76 ± 5, 2.5% CSE 142 ± 11 vs. controls 29 ± 6; $n = 3$, $P < 0.01$). This suggests that monocytes/macrophages secrete IL-8 and TNF-α protein in response to CSE. We also tested the possibility that the induction of CSE-mediated IL-8 release was due to the presence of endotoxin in the culture medium. Polymyxin B sulfate, an endotoxin binding agent, at a concentration of 10 μg/ml lowered the basal and stimulated IL-8 release equally without affecting the CSE-mediated IL-8 release, and therefore endotoxin does not play a role in the CSE-induced IL-8 release observed (data not shown). Measurement of lactate dehydrogenase (LDH) leakage from the cells demonstrated that 1% and 2.5% CSE concentrations were not toxic to the cell either at 4 h (% LDH release: 1% CSE 92 ± 5, 2.5% CSE 110 ± 10, 5% CSE 120 ± 26 vs. 100% control lysis; $n = 3$) or at 24 h (Fig. 2). However, CSE at a concentration of 5% resulted in significant LDH release ($P < 0.001$).

CSE Decreases Intracellular Levels of GSH

We investigated the oxidative effect of CSE on the levels of intracellular GSH in MonoMac6 cells. CSE dose-dependently decreased intracellular GSH concentrations, suggesting that CSE-mediated IL-8 release may be mediated, at least in part, by depletion of intracellular GSH levels (Fig. 3). Furthermore, when we studied the effect of DL-buthionine-[*S*,*R*]-sulfoximine (BSO; 50 and 100 μM), an inhibitor of glutamate cysteine ligase thereby depleting GSH, we found that depletion of GSH was associated with significant IL-8 release (pg/ml: control 20 ± 1.5, 50 μM BSO 325 ± 8.5, 100 μM BSO 295 ± 4.3; $n = 3$, $P < 0.001$). However, in our study CSE treatment did not change GSSG concentration in MonoMac6 cells either at 4 h (1% CSE 2 ± 0.5, 2.5% CSE 1.8 ± 1.0, 5% CSE 2.0 ± 0.6 vs. control 2.2 ± 1.0; $n = 3$, $P > 0.05$) or at 24 h (1% CSE 1.2 ± 0.05, 2.5% CSE 1.0 ± 0.4, 5% CSE 1.4 ± 0.2 vs. control 1.6 ± 0.8 nmol/mg protein; $n = 3$, $P > 0.05$).

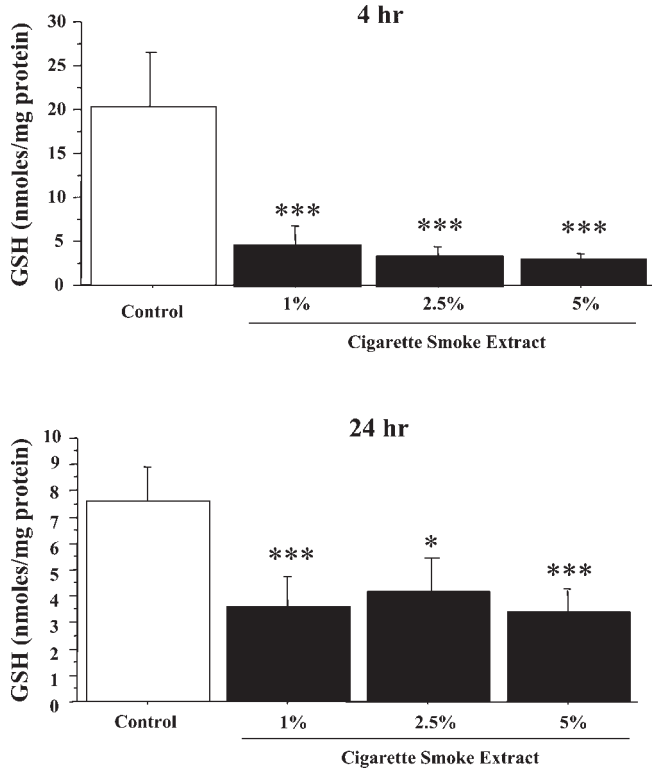

Fig. 3. CSE depletes intracellular glutathione (GSH) levels in a dose- and time-dependent manner in MonoMac6 cells. Intracellular GSH levels were depleted in MonoMac6 cells after treatment with CSE (1%, 2.5%, and 5%) at 2 different time points (4 and 24 h). Data are expressed as means of 3–4 experiments. $*P < 0.05$, $***P < 0.001$ compared with control values.

Thiol Compound GSHMEE but Not TR/TRX Inhibited CSE-Mediated IL-8 Release

To determine the role of intracellular thiol compounds on CSE-mediated increase in IL-8 release, we pretreated MonoMac6 cells with GSHMEE (to increase intracellular levels of thiols) (31) and TRX/TR. GSHMEE significantly inhibited CSE-mediated IL-8 release (Fig. 4). In contrast, pretreatment with TRX/TR did not inhibit the IL-8 release (pg/ml: 1% CSE 512 ± 47, 2.5% CSE 448 ± 125, TRX/TR 91 ± 16, CSE1% ± TRX/TR 535 ± 45, CSE 2.5% + TRX/TR 488 ± 53 vs. control 62 ± 4; $n = 3$, $P > 0.05$). This suggests that intracellular GSH, but not TRX, protects the cell from CSE-mediated induction of IL-8 release. We further examined the levels of intracellular GSH in response to GSHMEE and TRX/TR treatment in MonoMac6 cells. As expected, the increase in GSH level was more dramatic in GSHMEE (1 mM)-treated cells compared with TRX (1 μM)/TR (0.1 μM)-treated cells at 4 h (nmol/μg protein: control 15.4 ± 3.6, GSHMEE 120 ± 16, TRX/TR 62 ± 18) and 24 h (nmol/μg protein: control 28 ± 7, GSHMEE 143 ± 22, TRX/TR 58 ± 12) ($n = 3$; $P < 0.001$ for GSHMEE and $P < 0.01$ for TRX/TR vs. controls). CSE-mediated depletion of GSH (nmol/μg protein; $n = 3$) was more pronounced in TRX/TR+5% CSE (6.8 ± 2.1; $P < 0.01$)- than GSHMEE+5% CSE (68 ± 12)-treated cells compared with controls (15.4 ± 3.2) and 5% CSE (4.6 ± 1.2; $P < 0.001$). However, we did not find any significant increase in GSSG levels in response to these treatments.

CSE Induces Generation of Intracellular ROS

One of the aims of the study was to determine whether cigarette smoke differentially causes oxidative stress in MonoMac6 cells, in which we used ROS generation as the primary indicator. Cell populations of 2.5×10^5 were seeded in six-well plates and treated with cigarette smoke for 4, 12, and 24 h. Cells were washed with PBS and incubated with H_2DCFDA dye for 30 min. After harvesting, cell lysates were resuspended in PBS and ROS was measured by flow cytometer. Our data clearly show that CSE dose-dependently increased ROS production at 4, 12, and 24 h (Fig. 5). At 12 h, the increase was significant compared with 24 h, whereas ROS generation was not significant at 4 h of treatment. This suggests that the CSE-mediated proinflammatory effect is mediated via intracellular ROS generation in addition to its direct depletion of GSH by electrophilic conjugation.

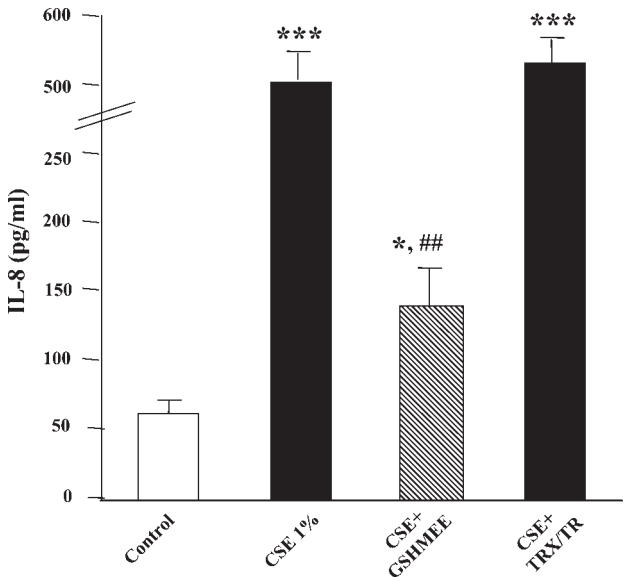

Fig. 4. Glutathione monoethyl ester (GSHMEE) pretreatment blocks CSE-mediated IL-8 release in MonoMac6 cells. Cells were pretreated with GSHMEE (1 mM) or thioredoxin (1 μM)/thioredoxin reductase (0.1 μM) (TRX/TR) for 2 h, washed, and then incubated with CSE 1%, and IL-8 was measured by ELISA. GSHMEE, but not TRX/TR, pretreatment protected cells against cigarette smoke-mediated IL-8 release. Results are an average of 3 experiments. $*P < 0.05$, $***P < 0.001$ compared with control values; $\#\#P < 0.01$ compared with CSE (1%).

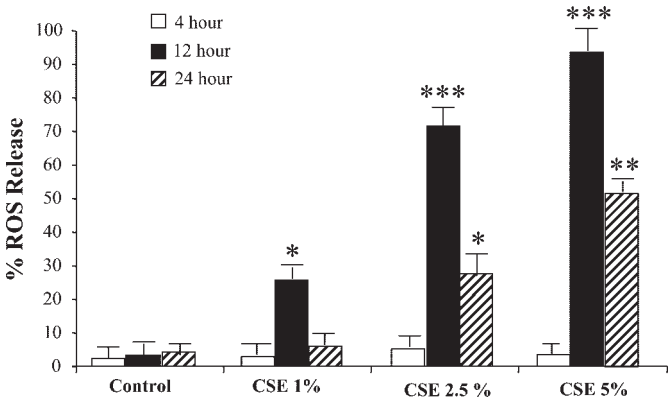

Fig. 5. CSE induces generation of reactive oxygen species (ROS) in MonoMac6 cells. Cells exposed to increasing concentrations of CSE (1%, 2.5%, and 5%) were analyzed for generation of ROS at 4, 12, and 24 h by the oxidation of 5-(-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate dye (H_2DCFDA, 10 μM). CSE dose- and time-dependently increased ROS production in MonoMac6 cells. Percent ROS release from a density plot of propidium iodide (viable cells) and H_2DCFDA double-stained cells measured by flow cytometer is shown. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ ($n = 3$).

Mechanism of Action of Cigarette Smoke on Nuclear Signaling

CSE decreases HDAC activity and HDAC1, HDAC2, and HDAC3 levels. To determine whether decreased histone deacetylation is involved in elevated IL-8 release in MonoMac6 cells, we measured HDAC activity with the Color de Lys substrate and the levels of HDAC1, HDAC2, and HDAC3 protein in response to CSE and TSA in these cells. CSE (1%,, 2.5% and 5%) significantly decreased HDAC activity (Fig. 6), which was associated with the decreased levels of HDAC1, HDAC2, and HDAC3 protein levels at 4 and 24 h (Figs. 7 and 8). We further showed that CSE-mediated inhibition of HDAC1, HDAC2, and HDAC3 levels was restored by GSHMEE pretreatment at 4 h (Fig. 7) but not at 24 h, in particular at high concentrations of CSE (Fig. 8), suggesting that electrophilic agents present in CSE can directly affect the HDAC proteins by covalent modifications of the HDAC enzymes and possibly detoxification of CSE components at an earlier time point (4 h).

Cigarette smoke covalently modifies HDAC proteins. In view of the prooxidative components present in cigarette smoke and the earlier reported ability of cigarette smoke to increase lipid peroxidation (46) as well as our observations of depletion of GSH-associated increased ROS release, we investigated whether protein modification is involved in the observed reduction in HDAC1, HDAC2, and HDAC3 levels.

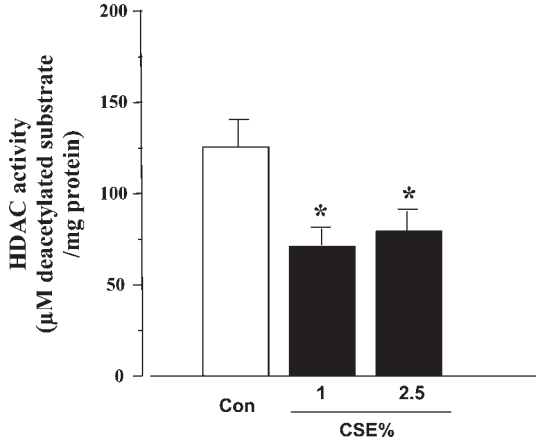

Fig. 6. CSE decreases histone deacetylase (HDAC) activity at 24 h in MonoMac6 cells. HDAC activity was measured with a colorimetric assay kit (Biomol) at 405 nm using Color de Lys substrate (500 μM). HeLa cell nuclear extract was used as a positive control. A standard curve was prepared using the known amount of the deacetylated standard included in the kit. HDAC levels were significantly decreased by CSE. *$P < 0.05$ compared with control values ($n = 3$).

Covalent modification of the HDAC proteins was assessed by immunoprecipitation, followed by Western blot analysis using monoclonal antibodies for 4-HNE and nitrated tyrosine. There was a significant increase in the tyrosine nitration and 4-HNE modification of HDAC1, -2, and -3 after 24 h of cigarette smoke exposure compared with untreated cells (Fig. 9). This supports the concept that the decrease in HDAC1, -2, and -3 levels by cigarette smoke exposure was in part due to the covalent modification of HDAC proteins by components of cigarette smoke. We further examined whether HDAC2 protein is associated with p65/RelA because it is known that glucocorticoids recruit HDAC2 in NF-κB transcriptional activation complex. Depletion of HDAC2 may be associated with activation of p65/RelA or vice versa. Immunoprecipitation of HDAC2 (20 μg) and Western blotting of NF-κB p65/Rel A revealed that p65/Rel A forms a complex with HDAC2 in nuclear extracts of cigarette smoke-exposed MonoMac6 cells (Fig. 10). Cigarette smoke treatment dose-dependently decreased the HDAC2 level, which was associated with increased p65 levels in the nucleus. A similar result was obtained for HDAC3, but not HDAC1, interaction with p65/RelA (unpublished observation). Decreased levels of HDAC2 were associated with modification by reactive aldehydes and nitric oxide products. This suggests that HDAC2 regulates the p65 subunit of NF-κB in the nucleus and HDAC2 protein is modified by posttranslational mechanisms.

Implication of CSE on NF-κB Activation and IL-8 Release

Because NF-κB is known to possess an intrinsic histone acetyl transferase activity (20), we examined the activation of NF-κB in response to CSE in MonoMac6 cells transiently transfected with an NF-κB-dependent promoter. TNF-α treatment was used as a positive control. CSE at various doses (1% and 2.5%) and TNF-α treatments significantly increased NF-κB *trans*-activation compared with the control values at 24 h (Fig. 11). However, CSE at a higher concentration (5%)

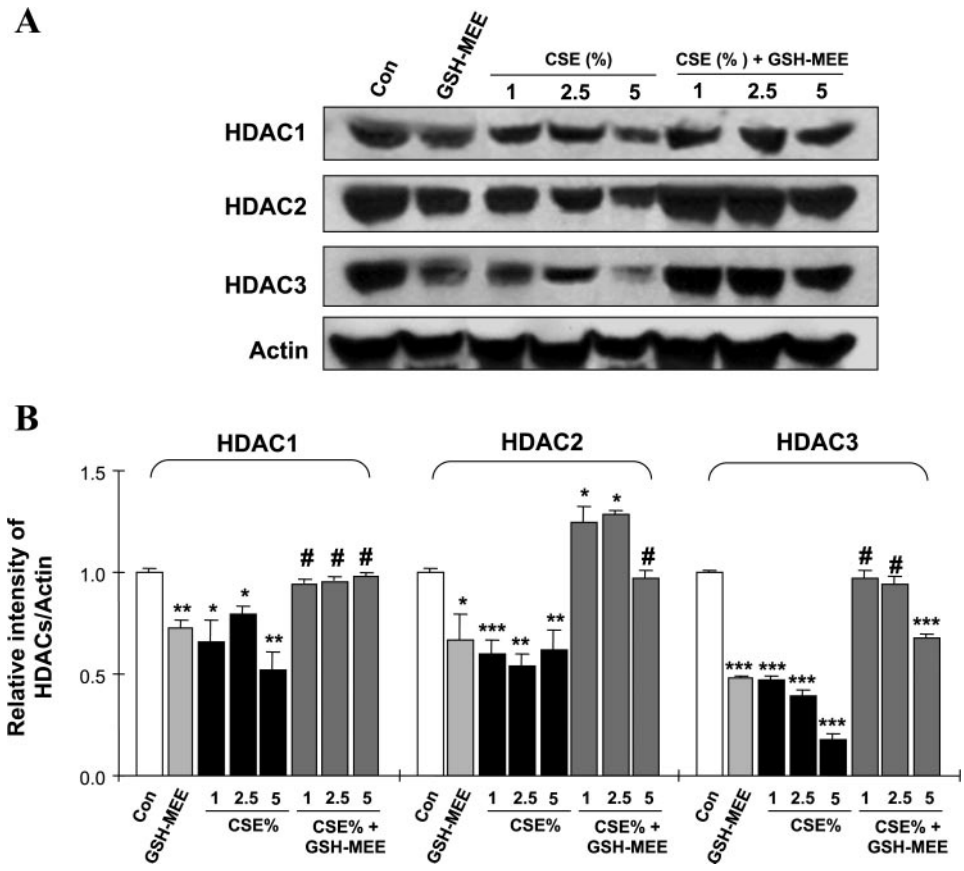

Fig. 7. CSE decreases HDAC1, -2, and -3 protein levels at 4 h. *A*: Western blots of soluble nuclear proteins extracted from treated MonoMac6 cells electrophoresed on 7.5% PAGE gels and electroblotted onto nitrocellulose membranes showing reduced levels of HDAC1, HDAC2, and HDAC3 proteins in response to CSE dose dependently at 4 h. Pretreatment of cells with GSHMEE (1 mM) for 4 h attenuated CSE depleted HDAC1, -2, and -3 levels (*B*). Gel pictures shown are representative of at least 3 separate experiments. *B*: relative density (% of control) of HDAC1, HDAC2, and HDAC3 in MonoMac6 cells after treatments described in *A* for 4 h alone and in combination with GSHMEE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control values; #not significant.

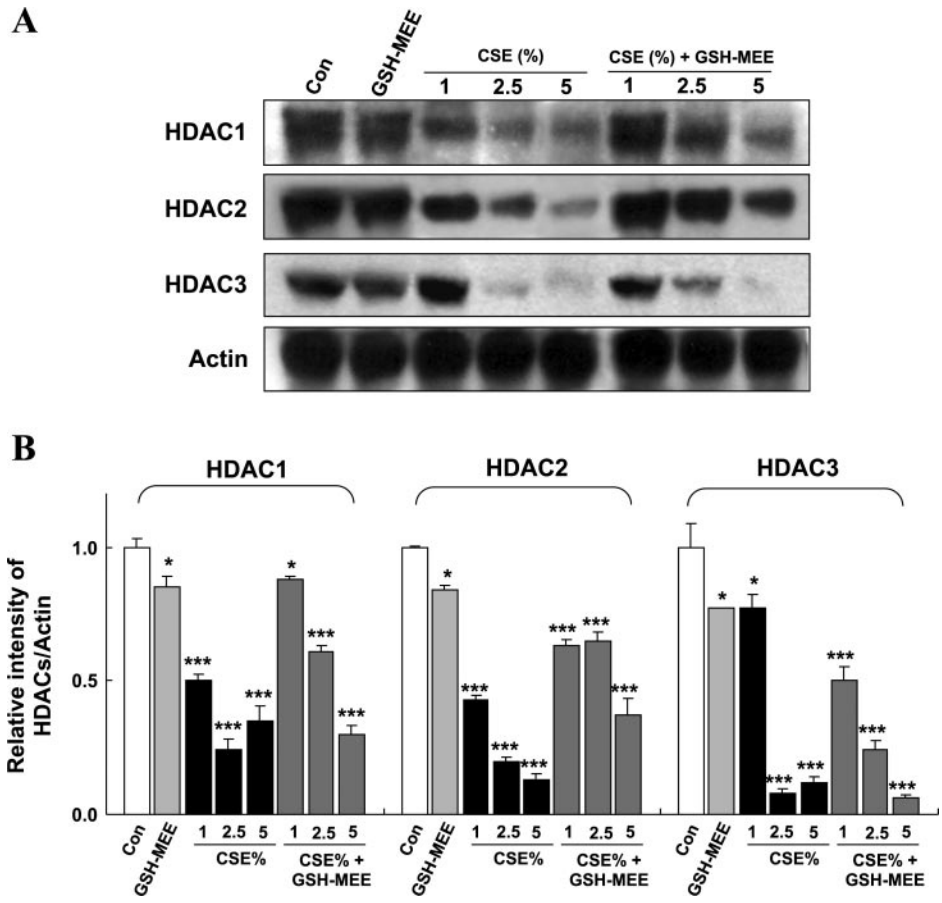

Fig. 8. CSE decreases HDAC1, -2, and -3 protein levels at 24 h. *A*: Western blots of soluble nuclear proteins extracted from treated MonoMac6 cells electrophoresed on 7.5% PAGE gels and electroblotted onto nitrocellulose membranes showing reduced levels of HDAC1, HDAC2, and HDAC3 proteins in response to CSE dose dependently at 24 h. Pretreatment of cells with GSHMEE (1 mM) did not significantly attenuate CSE-mediated depletion in HDAC protein levels at 24 h in response to GSHMEE pretreatment. Gel pictures shown are representative of at least 3 separate experiments. *B*: relative density (% of control) of HDAC1, HDAC2, and HDAC3 in MonoMac6 cells after treatments described in *A* for 24 h alone and in combination with GSHMEE. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, compared with control values.

did not activate NF-κB at 24 h (5% CSE 0.09 ± 0.05 vs. control 0.05 ± 0.04 relative luciferase unit; $n = 3$). Inhibition of HDAC by TSA also caused increased NF-κB transactivation in these cells (data not shown). To investigate whether CSE-mediated IL-8 release is dependent on NF-κB/IKK, we pretreated MonoMac6 cells with a specific inhibitor of IKK (IKKi; an analog of UK-436303) (30) and a commercial IKKi (BAY-117082) and assessed the effect of CSE on IL-8 release. We found that IKKi (whole cell IC_{50} 2 μM) significantly inhibited CSE- and TNF-α-mediated IL-8 release (Fig. 12*A*). Inhibition of NF-κB by IKKi (BAY-117082, 20 μM) showed similar results (Fig. 12*B*). This suggests that CSE-mediated induction of IL-8 release is dependent on NF-κB activation.

Further activation of NF-κB by CSE was confirmed by electrophoretic mobility shift assay. Treatment with CSE (1%, 2.5%, and 5%) for 4 h increased NF-κB nuclear binding in MonoMac6 cells (Fig. 13). Similar results were obtained when the cells were treated with TNF-α (10 ng/ml) as a positive control, water (negative control), and HeLa nuclear extract (positive control), and a cold competitor using 100-fold molar excess of unlabeled oligonucleotides showed competition with the bands (data not shown).

DISCUSSION

Lung macrophages are considered to be an important component in perpetuating the inflammatory responses to cigarette smoke (2, 50). We examined the effect of CSE on the release of inflammatory cytokines by human macrophage-like cells. We showed that CSE exposure at doses of 1% and 2.5% increased IL-8 release from MonoMac6 cells. However, at higher doses (5%), CSE did not increase IL-8 release. This may be related to cell cytotoxicity seen as increased LDH release at 24 h in 5% CSE exposure, whereas treatment of cells with 1% and 2.5% CSE did not show any significant LDH release. Normalizing percentage of live cells vs. dead cells and relating to IL-8 release, we found that CSE dose-dependently increased IL-8 release from these cells. Several studies have previously shown the importance of oxidative stress in the upregulation of various cytokines and chemokines, especially IL-8 (38, 47). Our data showing increased IL-8 release by treatment with CSE are consistent with the release of TNF-α and IL-8 by alveolar macrophages from normal subjects after exposure to tobacco smoke (10). Keatings and colleagues (23) showed high concentrations of IL-8 in induced sputum of COPD patients compared with healthy smokers and nonsmokers. Similarly, elevated levels of proinflammatory cytokines have been noted in bronchoalveolar lavage fluid obtained from COPD patients (35). It has also been reported that alveolar macrophages, as resident inflammatory cells, are a potential source of IL-8 after acute cigarette exposure (32, 33). The increased levels of proinflammatory cytokines within the lung compartment are speculated to be a contributing factor to the more severe inflammatory responses observed in COPD patients (14).

Cigarette smoke-mediated ROS activate transcription factors, including NF-κB and AP-1, and regulate expression of inflammatory genes such as IL-8 and TNF-α (39). CSE-

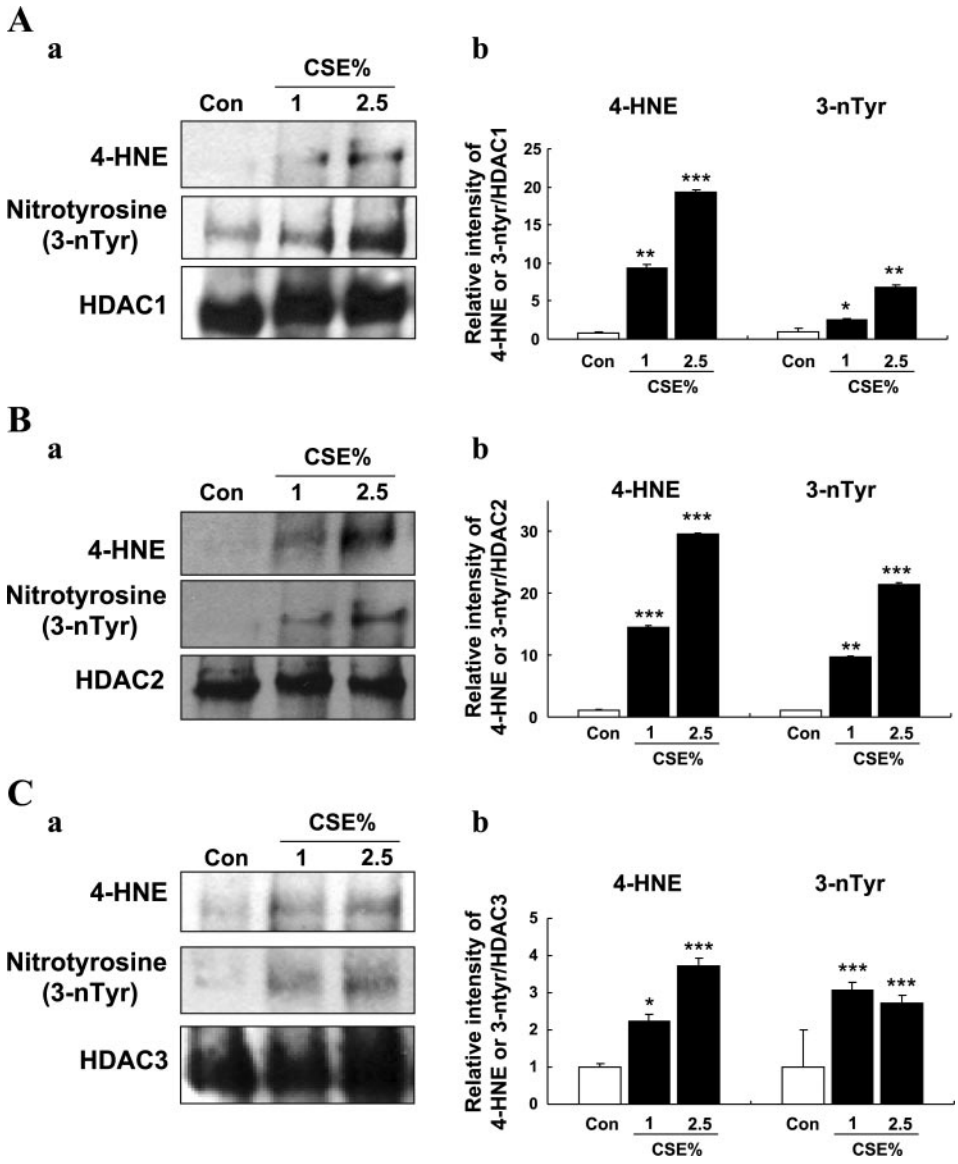

Fig. 9. CSE causes posttranslational modifications of HDAC proteins. *A*: 4-hydroxy-2-nonenol (4-HNE) and tyrosine nitration modification of HDAC1 protein is increased dose-dependently after cigarette smoke exposure in MonoMac6 cells compared with controls. *a*: Levels of 3-nitrotyrosine (3-nTyr)-HDAC1 adduct formation were much higher than 4-HNE-HDAC1 adducts. *b*: Histograms are expressed as relative intensity of HDAC1 protein bands. *B*: 4-HNE and tyrosine nitration modification of HDAC2 protein is increased dose-dependently after cigarette smoke exposure in MonoMac6 cells compared with controls. *a*: CSE (1% and 2.5%) significantly increased 4-HNE and 3-nitrotyrosine-HDAC2 adducts. *b*: Histograms are expressed as relative intensity of HDAC2 protein bands. *C*: 4-HNE and tyrosine nitration modification of HDAC3 protein is increased dose-dependently after cigarette smoke exposure in MonoMac6 cells compared with control. *a*: CSE (1% and 2.5%) significantly increased 4-HNE and 3-nitrotyrosine-HDAC2 adducts. *b*: Histograms are expressed as relative intensity of HDAC3 protein bands. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with control values.

induced IL-8 release may therefore be mediated through redox signaling pathways, particularly the activation of NF-κB. In support of this we have demonstrated an enhancement of NF-κB-driven luciferase activity and increased NF-κB DNA binding by gel shift assay in response to CSE treatment. Thus our data suggest that CSE-mediated release of IL-8 release may be mediated by NF-κB activation in MonoMac6 cells. This is supported by reports from other studies showing activation of NF-κB by cigarette smoke in Swiss 3T3, human histiocytic lymphoma U-937, and Jurkat T cells (1, 12, 26, 34). Our data also showed that CSE-mediated IL-8 release was dependent on NF-κB/IKK because inhibition of IKK2 by two specific IKKi (an analog of UK-436303 and BAY-117082) caused ablation of CSE-mediated IL-8 release in MonoMac6 cells. This confirms that CSE-mediated induction of IL-8 release is dependent on NF-κB activation.

Oxidative stress is thought to activate intracellular redox signaling pathways leading to the activation of transcription factors NF-κB and AP-1 (42). In this study, we show that CSE decreases GSH levels and this is associated with the activation of NF-κB/IL-8 release in MonoMac6 cells. CSE-mediated depletion of GSH was not associated with increased GSSG levels. We also found that depletion of GSH was associated with significant IL-8 release. We further demonstrate that CSE dose-dependently increased ROS production measured by flow cytometry using H_2DCFDA dye in MonoMac6 cells. This suggests that CSE induces its effects via intracellular ROS generation in addition to its direct electrophilic ability to deplete intracellular GSH. Our finding is supported by earlier studies showing that cigarette smoke induces oxidative stress by generation of ROS (15) and by decreasing intracellular GSH levels in alveolar type II cells (40). Thus it is conceivable that CSE generates ROS that may deplete GSH levels or, alternatively, electrophilic compounds present in cigarette smoke may form conjugate/adducts with intracellular GSH, leading to its depletion in MonoMac6 cells (40). Furthermore, our data suggest that the CSE-mediated proinflammatory effect was due to both oxidative and electrophilic covalent conjugation of thiols.

The antioxidant GSHMEE, which increased intracellular levels of GSH, was effective in reducing IL-8 release in

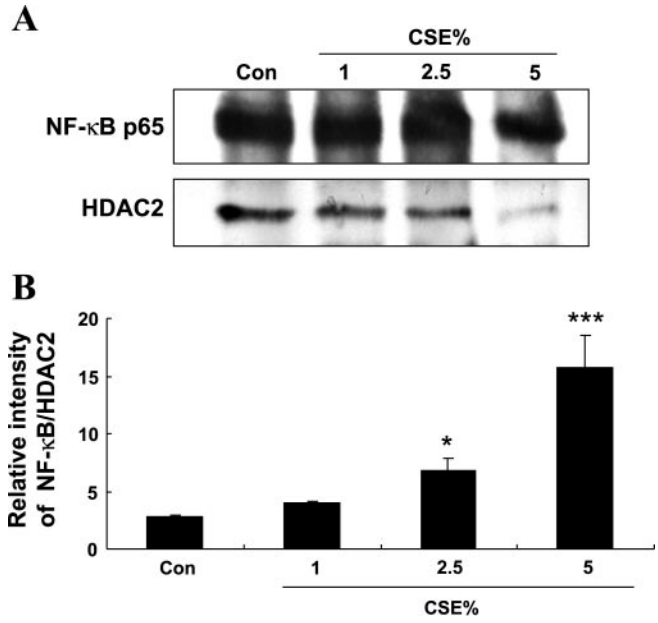

Fig. 10. HDAC2 interacts with p65/RelA of NF-κB in the nucleus. *A*: immunoprecipitation of HDAC2 and Western blotting of p65 NF-κB revealed that p65 forms a complex with HDAC2 in nuclear extracts of CSE-exposed MonoMac6 cells. *B*: CSE treatment dose-dependently decreased HDAC2 level, which was associated with increased p65/RelA levels in the nucleus. Equal amounts (20 μg) of immunoprecipated HDAC2 protein were used for Western blots. $*P < 0.05$, $***P < 0.001$ compared with control values.

response to cigarette smoke exposure, suggesting that GSH protects against the proinflammatory effects of CSE in MonoMac6 cells. We further tested whether another thiol-containing agent/system, the thioredoxin system (TRX and TR with NADPH, which is an ubiquitous oxidoreductase system with a thiol-dependent redox regulatory role), can attenuate CSE-induced IL-8 release. Surprisingly, our data show no effect of

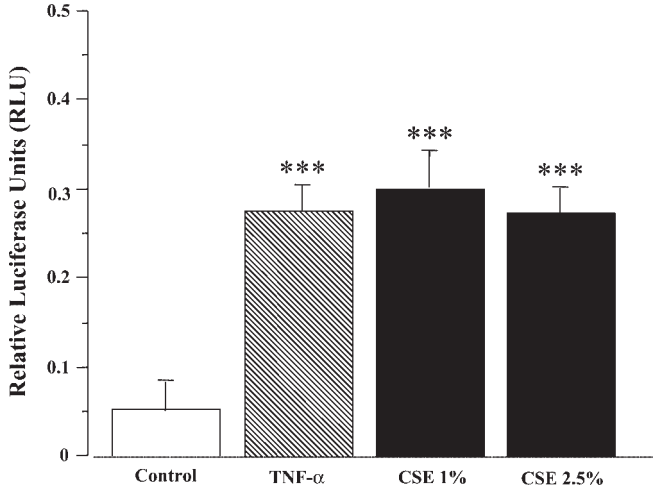

Fig. 11. CSE induces NF-κB-driven luciferase activity in transiently transfected MonoMac6 cells. NF-κB luciferase plasmid (2 μg DNA/well; 10-to-1 ratio) was mixed with Lipofectamine 2000 reagent in serum-free medium and transfected in MonoMac6 cells. MonoMac6 cells were treated with CSE (1% and 2.5%) and TNF-α as a positive control (10 ng/ml) for 24 h, and cell lysates were used to measure luciferase activity with a luminometer. The results show that CSE significantly increased the NF-κB luciferase activity (TNF-α used as a positive control). $***P < 0.001$ compared with control values ($n = 3$).

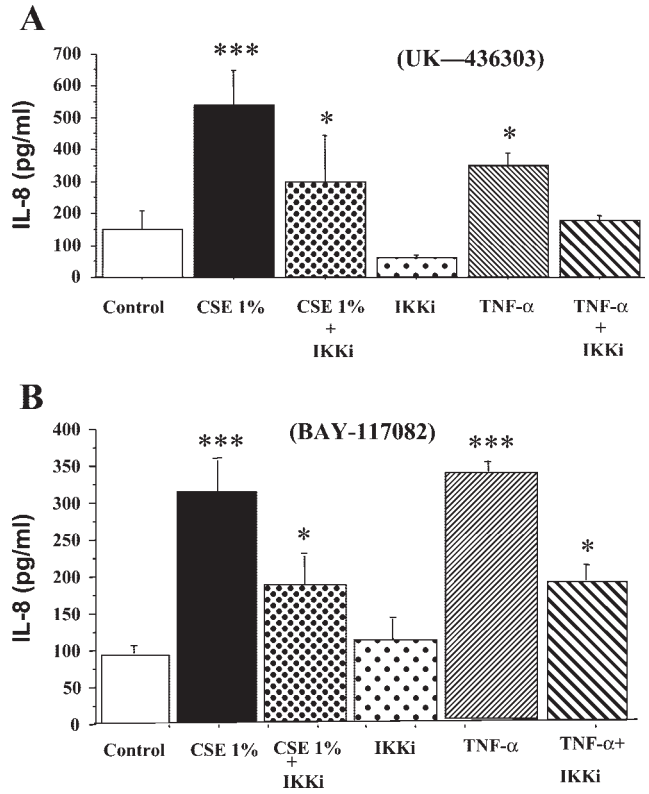

Fig. 12. Effect of NF-κB/IKK2 inhibitor (IKKi) on CSE-mediated IL-8 release in MonoMac 6 cells. Inhibition of IKK2 by two specific inhibitors, UK-436303 (2 μM; *A*) and BAY-117082 (20 μM; *B*), significantly inhibited both CSE- and TNF-α-induced IL-8 release, suggesting that CSE mediated IL-8 release was via IKK/NF-κB activation. Histograms represent means of 3–6 experiments.$*P < 0.05$, $***P < 0.001$ compared with control values ($n = 3$–6).

pretreatment with TRX/TR on CSE-induced IL-8 release. The reason for the lack of inhibition of CSE-induced IL-8 release by TRX/TR is not known. It is possible that higher levels of reduced TRX/TR may be required to have any inhibitory effect on deleterious effects of CSE or that electrophilic compounds present in CSE form conjugates with the sulfydryl group of GSH, whereas the thiol groups present on TRX/TR are only effective with oxido-reduction/thiol-dependent enzymatic reactions. However, more studies are required to confirm these contentions.

The family of HDAC enzymes consists of 17 isoforms grouped into three classes (8). Whereas the class I HDACs (HDAC1, -2, -3, -8, and -11) reside almost exclusively in the nucleus, the class II HDACs (HDAC4, -5, -6, -7, -9, and -10) are able to shuttle between the nucleus and cytoplasm in response to certain cellular signals. The third HDAC class are NAD^+ dependent, consisting of sirtuins 1–6. The functions of sirtuins 1–6 are not yet fully deciphered, but they are thought to be important in aging/senescence and often prefer nonhistone proteins as substrates. Various members of the class I HDAC family have been shown to play a role in the regulation of cell proliferation and inflammatory responses (8). Recently, HDAC2 has been reported to be required for corticosteroid-mediated anti-inflammatory activity (22). Furthermore, there is increasing evidence to suggest that HDAC1 and HDAC3 play an important role in regulating proinflammatory responses by directly or indirectly modifying the nuclear activity of tran-

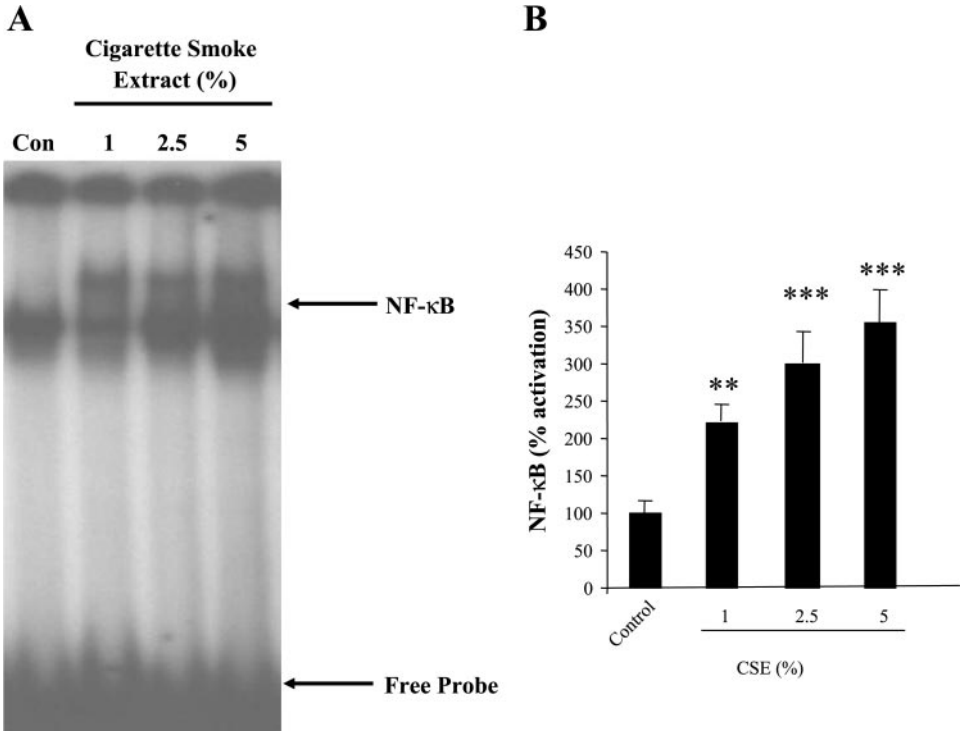

Fig. 13. Effect of CSE on NF-κB DNA binding measured by electrophoretic mobility shift assay in MonoMac 6 cells. *A*: electrophoretic mobility shift assay of nuclear extracts isolated from MonoMac6 cells showing nuclear binding of NF-κB after CSE treatments. Nuclear extracts were prepared and analyzed by electrophoretic mobility shift assay using γ-^{32}P-labeled NF-κB oligonucleotide. The DNA-protein complexes formed are indicated (arrow) by NF-κB specific band after exposure of MonoMac6 cells to CSE (1%, 2.5%, and 5%) and TNF-α for 4 h. Autoradiographs shown are representative of at least 3 experiments. *B*: densitometric quantitation of specific NF-κB binding was compared with the control values set at 100%. Histograms represent mean values and error bars SE of the relative intensity of the bands of 3 experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control values.

scription factors such as NF-κB (4, 53). Histone deacetylation represses gene transcription by limiting access to transcription factors in the coactivator complex. It has been shown that chemical inhibition of HDAC enhances TNF-α-mediated NF-κB DNA binding and transcriptional activation of an NF-κB-luciferase reporter construct in a number of cell lines compared with TNF-α alone (5, 17, 41, 53). In this study, for the first time, we show that CSE-mediated IL-8 release was associated with a reduction in HDAC activity, particularly the levels of HDAC1, -2, and -3. This suggests that CSE-mediated IL-8 release is associated with both NF-κB activation and decreased HDAC activity/levels. We further show that CSE-mediated reduction in HDAC2 was associated with increased p65/Rel A. This clearly suggests that p65/RelA interacts with HDAC2 and when HDAC2 is decreased p65/RelA becomes available or retained in the nucleus for proinflammatory gene transcription (5, 53). Similar data were obtained for HDAC3, but not HDAC1, interaction with p65/RelA, suggesting an important role of HDAC2 and HDAC3 in regulation of NF-κB in the nucleus (unpublished observation). Ito and coworkers (21) have demonstrated a role for histone acetylation and deacetylation in IL-1β-induced TNF-α release in alveolar macrophages derived from cigarette smokers. They have also proposed that oxidants may play an important role in the modulation of HDAC activity and inflammatory cytokine gene transcription. HDAC activity has also been measured in bronchial biopsies and alveolar macrophages from COPD patients and smoking subjects, demonstrating a significant decrease in HDAC activity, the magnitude of which increases with severity of disease (19). Moreover, protein expression of HDAC2 and HDAC3 and mRNA expression of HDAC5 and HDAC8 were decreased in a similar manner in COPD patients. Our observation of CSE-mediated inhibition of HDAC activity and HDAC protein levels in MonoMac6 cells is consistent with these results and the earlier reports of reduced levels of HDAC2 in peripheral lung tissue of smokers and patients with COPD (6, 7, 18, 21). However, the molecular mechanism of reduction of HDAC proteins was not investigated.

CSE-mediated reduction in HDAC activity was associated with a decrease in HDAC1, HDAC2, and HDAC3 levels. CSE may inhibit HDAC expression and/or activity by covalent modifications of amino acid residues present in the active site by reactive aldehydes and nitric oxide present in CSE (36) and subsequent degradation by the ubiquitin-proteasome pathway (8, 25). To prove this contention, we investigated the mechanism of the observed alteration in HDAC levels by studying the protein modifications of HDAC1, -2, and -3 by CSE exposure in MonoMac6 cells. We show that a posttranslational modification of HDAC1–3 is due to increased covalent modification by 4-HNE and increased tyrosine nitration after CSE exposure. This is not surprising, because HDAC enzyme contains lysine and histidine residues in the active site (11). These groups are labile for modifications by alkylating or oxidizing agents.

4-HNE, a reactive aldehyde, can be ubiquitously generated in biological systems under oxidative stress as a product of lipid peroxidation reactions and is a known marker of oxidative stress (9, 46). It can react with cysteine, histidine, and lysine residues and therefore may be considered an important mediator of cell damage by disruption of protein function through covalent modification. As the active site of HDAC contains histidine groups (11), the increased 4-HNE modifications recorded after smoke condensate exposure may therefore form a part of the mechanism responsible for the decrease in HDAC activity.

Tyrosine nitration of proteins also significantly disrupts protein function and turnover (16). Posttranslationally modified proteins such as HDAC and IκBα can be the targets of proteolytic degradation and removal (9). The increased HDAC

protein tyrosine nitration seen after CSE exposure may trigger increased proteolytic degradation of this protein, resulting in decreased HDAC levels as well as lowered HDAC activity. Thus our data showing posttranslational modification of HDAC1–2 (aldehyde-adduct formation and tyrosine nitration) and previous data showing the importance of this enzyme (particularly HDAC2) in the mechanism of action of steroids may explain at least part of the mechanism for poor glucocorticoid anti-inflammatory efficacy in response to smoking (21).

Our data showing increased intracellular thiol levels attenuating the CSE-mediated decrease in HDAC levels/activity suggest that the HDAC enzyme is redox regulated. This is supported by the findings that increase in intracellular thiol status significantly restored CSE-mediated reduction in HDAC1, -2, and -3 levels at 4 h but not at 24 h, in particular at high concentrations of CSE. This implies that restoration of HDAC levels was mainly due to thiol nucleophilic conjugation with electrophilic components of CSE and possibly inhibiting ROS release at an earlier time point. However, this thiol-reversing effect on HDAC proteins was not significant at 24 h of CSE treatments, particularly at the 5% CSE concentration, suggesting that the intracellular thiol pool was not quite enough at 24 h to nullify the sustained effect of the high dose of CSE. It may be important to mention here that other detoxification processes such as denitration by denitrases and reduction of aldehyde/carbonyl groups by aldoreductases/aldehyde dehydrogenase may be necessary for reversing the posttranslationally modified HDAC proteins. This certainly requires further studies.

In conclusion, the present study provides novel data on an important molecular mechanism by which cigarette smoke induces gene transcription and augments an inflammatory response in macrophages. CSE causes a decrease in HDAC activity and HDAC1, HDAC2, and HDAC3 levels and upregulates NF-κB-dependent proinflammatory cytokine release in MonoMac6 cells. Inhibition of IKK ablated the CSE-mediated IL-8 release, suggesting this process is dependent on the NF-κB pathway. Decreased HDAC activity was associated with depletion of intracellular GSH and may at least in part be due to modification of HDAC1, HDAC2, and HDAC3 by reactive aldehydes and nitric oxide products present in cigarette smoke. This may be important in amplification of the chronic inflammatory response induced by cigarette smoke in the lungs. Elevation of intracellular GSH restored HDAC activity/levels and abolished CSE-mediated IL-8 release, suggesting that reversal of oxidative stress with thiol antioxidants can be useful in therapeutic approaches to modulate intracellular nuclear signaling in diseases with a high level of oxidative stress such as COPD. Moreover, cigarette smoke-mediated proinflammatory events are regulated by the redox status of the cells.

ACKNOWLEDGMENTS

We thank Dr. Arshad Rahman for helpful technical advice and support.

GRANTS

This study was supported by the National Institute of Environmental Health Sciences Environmental Health Sciences Center (EHSC) (ES-01247) and an EHSC Pilot Project fund.

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