ANTIOXIDANTS & REDOX SIGNALING
Volume 7, Numbers 1 & 2, 2005

Forum Original Research Communication

Inhibition of NFκB Activation and IL-8 Expression in Human Bronchial Epithelial Cells by Acrolein

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ABSTRACT

Lipid oxidation and environmental pollutants are major sources of α,β-unsaturated aldehydes such as acrolein and 4-hydroxynonenal. Acrolein (2-propenal), a major product of organic combustion such as tobacco smoke, represents the most reactive α,β-unsaturated aldehyde, with high reactivity toward nucleophilic targets such as sulfhydryl groups. To investigate how acrolein affects respiratory tract cell activation, we exposed either primary (NHBE) or immortalized human bronchial epithelial cells (HBE1) to 0–25 μ*M* acrolein, and determined effects on basal and tumor necrosis factor-α (TNFα)-induced production of the chemokine interleukin (IL)-8. Cell exposure to acrolein dose-dependently suppressed IL-8 mRNA levels in HBE1 cells (26, 40, and 79% at 5, 10, and 25 μ*M* acrolein concentrations, respectively) and resulted in corresponding decreases in IL-8 production. Studies of nuclear factor-κB (NFκB) activation, an essential event in IL-8 production, showed decreased TNFα-induced NFκB activation by acrolein, illustrated by inhibition of nuclear translocation of NFκB and reduced IκBα degradation. Immunochemical analysis of IκB kinase (IKK), a redox-sensitive regulator of NFκB activation, indicated direct modification of the IKK β-subunit by acrolein, suggesting that acrolein may act directly on IKK. In summary, our results demonstrate that acrolein can suppress inflammatory processes in the airways by inhibiting epithelial IL-8 production through direct or indirect inhibitory effects on NFκB activation. *Antioxid. Redox Signal.* 7, 25–31.

INTRODUCTION

ACROLEIN is a highly electrophilic α,β-unsaturated aldehyde (5, 38) produced in a variety of natural and synthetic processes, including incomplete combustion or pyrolysis of organic materials such as fuels, wood, synthetic polymers, food, and tobacco (9). It has been identified as a hazardous air pollutant of significant human concern, particularly as a component of industrial pollution and cigarette smoke (CS) (3). Many adverse cellular effects are observed following exposure to acrolein, including growth inhibition, increased cell membrane permeability, and the induction of apoptotic or necrotic cell death (14, 18, 25). In addition, acrolein exposure has been reported to affect inflammatory-immune processes by inhibiting the release of inflammatory cytokines such as interleukin (IL)-1β, IL-12, and tumor necrosis factor-α (TNFα) by, *e.g.,* human alveolar macrophages (20). Furthermore, acute exposure to CS inhibits the ability of bronchial epithelial cells to attach to the extracellular matrix and to migrate in response to chemotactic stimuli (6). Hence, alteration of epithelial cell repair processes could be one mechanism by which CS can affect normal airway function and structure (37).

A major signaling pathway involved in lung inflammation is mediated by the transcription factor nuclear factor-κB (NFκB) (29, 32). NFκB consists of heterodimers of two polypeptides, p50 and p65, which are members of a family of proteins related to the protooncogene c-rel. Inactive NFκB is located in the cytoplasm in a complex with its inhibitory protein, IκB.

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Activation of NFκB involves the phosphorylation, ubiquitination, and degradation of IκB, liberating NFκB and allowing its translocation into the nucleus, where it interacts with specific κB motifs in the promotor region of various target genes to regulate their transcription (2, 21, 33, 35). One of the genes that is regulated by NFκB is IL-8, a member of the CXC chemokine family that acts as a potent chemotactic factor and activator for neutrophils, T-lymphocytes, eosinophils, and monocytes (34), and plays an important role in airway disease associated with neutrophilia. Although several studies have suggested that unsaturated aldehydes are capable of modulating inflammation by suppressing NFκB signaling (16, 18), the mechanisms involved in such regulation are not clarified. In the present study, we investigated the effects of acrolein exposure on IL-8 chemokine production by tracheobronchial epithelial cells, and the potential involvement of alterations in NFκB signaling.

MATERIALS AND METHODS

Cell culture

Experiments were performed with papilloma virus-immortalized human bronchial epithelial cells from a normal subject (HBE1), used at passage 25–28 (39), and with primary normal human bronchial epithelial cells (NHBE) (Clonetics). HBE1 cells were cultured in serum-free Ham's F12 medium (GibcoBRL Life Technologies, Grand Island, NY, U.S.A.), containing penicillin (60 U/ml), streptomycin (60 μg/ml), gentamicin (50 μg/ml), insulin (5 μg/ml), transferrin (5 μg/ml), epidermal growth factor (20 ng/ml), dexamethasone (0.1 μ*M*), cholera toxin (20 ng/ml), and bovine hypothalamus extract (30 μg/ml) at 37°C (19). For experiments, cells (HBE1 and NHBE) were placed in six-well cell culture plates at a density of 75×10^3 cells/cm^2 and grown until subconfluence (~85%).

Cell treatment

Prior to treatments, cells were cultured in F12 medium without growth factors for 12 h, which did not result in significant loss of cell viability, as measured by lactate dehydrogenase leakage. Before exposure, cells were washed three times with phosphate-buffered saline (PBS), and subsequently treated for 30 min with 0–25 μ*M* acrolein (Aldrich Chemical, Milwaukee, WI, U.S.A.) in Hanks' balanced salt solution (Sigma, St. Louis, MO, U.S.A.). The acrolein doses used were based on reported concentrations in mainstream or secondhand tobacco smoke and on estimated concentrations in lung surface fluids upon inhalation (7, 8). Following acrolein treatment, cells were washed three times with PBS, placed in F12 medium without growth factors, and stimulated with 100 ng/ml TNFα. The medium was collected and frozen at −80°C for enzyme-linked immunosorbent assays (ELISA), and cells were harvested at the different time points following stimulation for analysis of NFκB activation or IL-8 expression.

ELISA detection of IL-8

Cellular production of IL-8 was measured by analysis of conditioned media using an ELISA kit according to the manufacturer's instructions (IL-8, R&D Systems, Minneapolis, MN, U.S.A.). The sensitivity of the assay enables detection of IL-8 levels as low as 3 pg/ml.

Western blot analysis of IκB

Cells were lysed in 60 μl of RIPA buffer (150 m*M* NaCl, 50 m*M* Tris-HCl, pH 7.4, 1% NP-40, 1 m*M* EDTA, 1 m*M* EGTA, 0.1% sodium dodecyl sulfate (SDS), 5 m*M* dithiothreitol (DTT), 5 m*M* NaF, 1 m*M* phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml iodoacetamide) (Sigma), and kept on ice for 1 h after the lysates were centrifuged at 15,000 rpm for 10 min and supernatants stored at −80°C until analysis. For western blot analysis, samples (40 μg of protein; determined using the Bio-Rad protein assay) were separated on 4–20% gradient SDS–polyacrylamide gel electrophoresis (PAGE) gels and electrotransferred onto nitrocellulose membranes. After blocking in 5% nonfat milk in PBS, the membranes were incubated with 1 μg/ml IκBα antibody (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.). Goat anti-rabbit horseradish peroxidase-conjugated antibody (Sigma) was used as secondary antibody, which was detected with enhanced chemiluminescence (ECL) (Enhanced Chemiluminescence Detection Kit, Amersham). Band densities were quantitated using NIH image shareware.

Electrophoretic mobility shift assay (EMSA)

HBE1 cells were plated on 100-mm dishes (7.5×10^5 cells/dish) and treated as described. To prepare nuclear extracts, cells were washed three times with ice-cold PBS, and dishes placed on dry ice/ethanol. Cells were lysed in 500 μl/dish buffer A (10 m*M* HEPES, pH 7.9, 10 m*M* KCl, 0.2 m*M* EDTA, 0.1 m*M* EGTA, 1 m*M* DTT, 1 m*M* PMSF, 1 m*M* leupeptin) and transferred to microcentrifuge tubes. After incubation for 10 min on ice, Nonidet P-40 (final concentration 0.02%) was added, and suspensions were passed six times through a 26.5-G needle to optimize cell lysis. Nuclei were pelleted by centrifugation for 2 min at 10,000 rpm, and supernatant (cytoplasmic extract) fractions were collected. The nuclear pellet was resuspended in 50 μl of buffer B (20 m*M* HEPES, pH 7.9, 0.4 *M* NaCl, 1 m*M* EDTA, 1 m*M* DTT, 1 m*M* PMSF, 1 m*M* leupeptin), incubated for 30 min on ice, and centrifuged for 5 min at 10,000 rpm at 4°C to obtain clear supernatant fractions (nuclear extract). An NFκB consensus oligonucleotide (5'–AGT TGA GGG GAC TTT CCC AGG C–3') and NFκB mutant oligonuleotide (5'–AGT TGA GGC GAC TTT CCC AGG C–3') (Santa Cruz Biotechnology) were labeled with digoxygenin (Dig) using a DIG Genius gel shift kit (Boehringer Mannheim, Indianapolis, IN, U.S.A.) and incubated overnight at 5°C with nuclear extract (20 μg of protein). The complexes were electrophoresed (0.5× Tris–borate–EDTA buffer, 150 V for 2 h at room temperature) on Novex 6% nondenaturing polyacrylamide gels (Invitrogen) and electroblotted (400 mA for 1 h) in a sandwich procedure onto nitrocellulose (protein) and nylon (oligonucleotide) membranes (28 and references therein). The Dig-labeled oligonucleotide was detected by anti-Dig alkaline phosphatase-conjugated Fab fragments and enhanced chemiluminescence. The p50 subunit of NFκB on the nitrocellulose membrane was probed by rabbit anti-NFκB (p50) antibody and detected by ECL Plus.

RNA extraction and reverse transcription–polymerase chain reaction (RT-PCR)

Total RNA was extracted from cells by addition of 0.5 ml/dish TRIZOL reagent (Life Technologies, Inc., Rockville, MD, U.S.A.), incubation at room temperature for 5 min, and chloroform (100 µl) extraction. The lysate was shaken vigorously for 15 s and centrifuged at 12,000 *g* at 4°C for 20 min. The top aqueous layer was transferred to a microcentrifuge tube containing 300 µl of isopropanol, mixed for 10 s, and centrifuged at 12,000 *g* at 4°C for 10 min. Pellets containing RNA were washed twice with 80% ethanol and once with 100% ethanol, dried at 70°C for 10 min, and resuspended with 20 µl of water. RT was performed on 2 µg of total RNA using Superscript II (Life Technologies) in a final volume of 20 µl, containing the following (final concentration): PCR buffer (1×), deoxynucleotide (0.2 m*M* each), $MgCl_2$ (2 m*M*), Taq DNA polymerase (2 U), oligonucleotide primer (0.2 *M*), and reverse transcriptase products. The following primers were designed based on cDNA sequences: IL-8, 5'-CGA TGT CAT GCA TAA AGA CA-3' (sense) and 5'-TGA ATT CTC AGC CCT CTT CAA AAA-3' (antisense); β-actin, 5'-TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA-3' (sense) and 5'-CTA GAA GCA TTT GCG GTG GAC GAT GGA GGG-3' (antisense) (Operon Technologies Inc., Alameda, CA, U.S.A.). One tenth of the cDNA was amplified PCR. The amplification conditions were the same for IL-8 and β-actin (35 cycles including a 2-min denaturation at 94°C, 45 s at 55°C annealing, and a 90-s extension at 72°C). Twenty microliters of each PCR reaction was separated on 2% agarose gels containing 0.2 µg/ml ethidium bromide. The gel was then photographed under ultraviolet light.

Immunoprecipitation and detection of IκB kinase β (IKKβ)

Cell lysates containing 500 µg of protein were mixed with protein A-Sepharose beads and 2 µg of a monoclonal antibody against IKKβ (Santa Cruz). Following immunoprecipitation of the IKK complex, the presence of acrolein-induced Michael adducts was determined by derivatization using dinitrophenylhydrazine (DNPH), after which proteins were separated by SDS-PAGE, electrotransferred to polyvinylidene difluoride membranes, and immunoblotted with an α-dinitrophenol (α-DNP) antibody (OxyBlot kit; Intergen, Purchase, NY, U.S.A.). In addition, IKKβ was immunoblotted using an antibody against IKKβ to verify the immunoprecipitation procedure.

RESULTS

Exposure to acrolein suppresses IL-8 production in airway epithelial cells

To study the effect of acrolein exposure on IL-8 expression at the protein level, HBE1 and NHBE cells were stimulated with TNFα, and IL-8 was determined in conditioned media by ELISA. Unstimulated HBE1 and NHBE cells were found to secrete detectable amounts of IL-8, and this was increased about two-fold following cell stimulation with 100 ng/ml TNFα (Fig. 1). Preexposure of cells up to 25 µ*M* acrolein significantly decreased basal secretion of IL-8 over 24 h, in both NHBE (45%) and HBE1 cells (12%). The inhibitory effect of acrolein was dose-dependent and more pronounced after cell stimulation with TNFα, in which case 25 µ*M* acrolein caused 62% and 50% reduction in IL-8 release by HBE1 and NHBE cells, respectively.

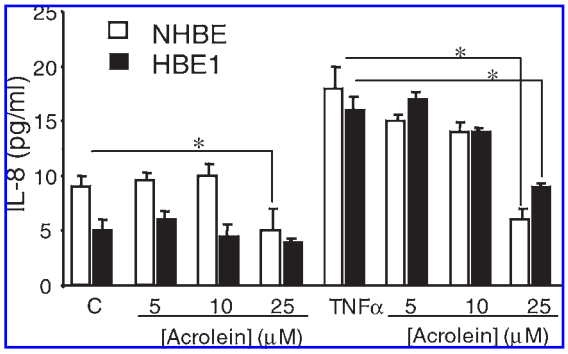

FIG. 1. Acrolein inhibits IL-8 secretion by tracheobronchial epithelial cells. HBE1 and NHBE cells were treated with acrolein for 30 min, at concentrations ranging from 0 to 25 µ*M*, followed by 30 min of stimulation with 100 ng/ml TNFα. IL-8 was measured in the culture after 24 h, and mean values ± SD are presented from three separate experiments. *Significant differences at $p < 0.05$.

Analysis of basal or TNFα-induced IL-8 mRNA expression by RT-PCR showed similar effects of acrolein exposure. As illustrated in Fig. 2, cell exposure to 5–25 µ*M* acrolein markedly decreased TNFα-induced IL-8 mRNA expression. Effects on basal IL-8 expression were again less pronounced, although 25 µ*M* acrolein significantly reduced basal IL-8 mRNA levels.

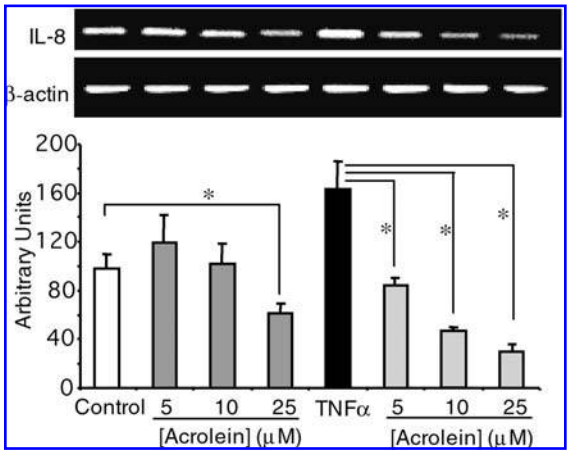

FIG. 2. Acrolein exposure inhibits IL-8 gene expression in tracheobronchial epithelial cells. HBE1 cells were treated with acrolein (0–25 µ*M*) for 30 min, followed by 30 min of stimulation with 100 ng/ml TNFα. Total RNA was extracted after 12 h, and IL-8 gene expression was determined by RT-PCR, using β-actin as an internal control. Mean values ± SD from three experiments are shown. *Significant differences at $p < 0.05$.

Collectively, these results indicate that airway exposure to acrolein may have antiinflammatory effects by reducing epithelial production of the potent neutrophil chemokine IL-8.

Acrolein inhibits NFκB activation in HBE1 cells

Activation of the transcription factor NFκB is a critical event in TNFα-mediated induction of many inflammatory genes, including IL-8 (23). We, therefore, investigated the effects of acrolein exposure on TNFα-induced NFκB activation, by EMSA analysis of activated NFκB in nuclear extracts. As shown in Fig. 3, TNFα stimulation caused a significant increase in NFκB activity, illustrated by increased electrophoretic mobility shift of Dig-labeled oligonucleotide. Analysis of the p50 subunit of NFκB that was simultaneously transferred onto nitrocellulose indicated that the observed Dig-labeled oligonucleotide comigrated with p50, as observed earlier in identical situations (28). The activation of NFκB was inhibited by acrolein exposure in a dose-dependent fashion. In addition, acrolein treatment also significantly inhibited basal NFκB activity at 25 μ*M*. These results suggest that acrolein exposure may suppress inflammatory cytokine production by inhibiting basal or TNFα-stimulated NFκB activation.

Effect of acrolein on cell IκBα levels

The activity of NFκB is controlled by IκBs, of which the best characterized member is IκBα (13), and IκB degradation is an essential step in the activation of NFκB. We examined the effect of acrolein exposure on IκBα levels in HBE1 cells, in untreated or TNFα-stimulated cells, by Western blotting. As shown in Fig. 4, acrolein exposure caused a dose-dependent increase in IκBα levels, determined 1 h after acrolein treatment and/or TNFα stimulation, suggesting that acrolein may prevent degradation of IκB and thereby inhibit NFκB activation.

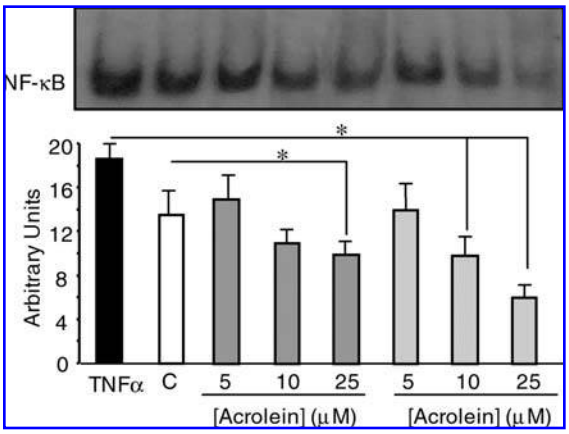

FIG. 3. Acrolein inhibits cytokine-induced activation of NFκB. HBE1 cells were incubated with the indicated concentrations of acrolein for 30 min, followed by 30 min of stimulation with 100 ng/ml TNFα. NFκB activation and nuclear translocation were determined by EMSA, using electrotransfer onto nylon and nitrocellulose membranes for immunodetection of Dig-labeled oligonucleotide and the p50 subunit of NFκB, respectively. (**Top panel**) Representative blot of Dig-labeled oligonucleotide that comigrated with p50. (**Bottom page**) Quantitative results (means ± SD) from three independent experiments.

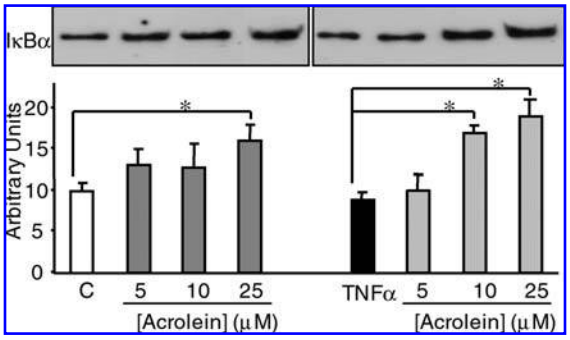

FIG. 4. Effect of acrolein on IκBα levels. HBE1 cells were incubated with acrolein for 30 min, followed by 30 min of stimulation with 100 ng/ml TNFα. Cytosolic fractions were obtained after 6 h, and separated on 4–20% SDS-PAGE gels (40 μg/lane), followed by western blot analysis for IκBα. (**Top panel**) Representative experiment. (**Bottom panel**) Data presented as the arithmetic mean ± SD from three independent experiments.

IKKβ: a target for alkylation by acrolein?

Based on recent findings that the β-subunit of IKK contains a redox-sensitive cysteine residue that is subject to oxidation or alkylation by various agents (16, 17, 31), we postulated that IKK might present a direct target for acrolein, and that alkylation of one or more IKK subunits by acrolein may prevent its activation and the subsequent activation of NFκB. To verify whether acrolein forms Michael adducts with IKKβ, we immunoprecipitated IKK from lysates of untreated and acrolein-exposed HBE1 cells, and derivatized immunoprecipitated proteins with DNPH (26). As illustrated in Fig. 5, increased immunoreactivity for DNP was observed in IKKβ obtained from acrolein-treated cells, consistent with direct modification of IKKβ by Michael addition, presumably on its redox-sensitive cysteine residue. By analogy, similar modification of IKK and inhibition of IKK activity have been proposed in response to 4-hydroxynonenal (4HNE) (16) or cyclopentenone prostaglandins (31), by similar alkylation of a cysteine residue in the activator loop of IKKβ.

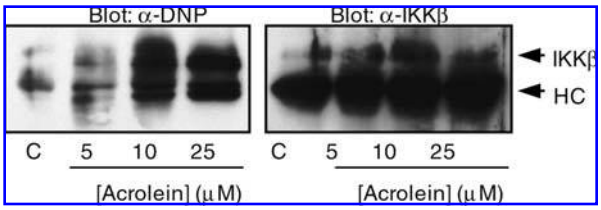

FIG. 5. Covalent modification of IKKβ by acrolein. HBE1 cells were treated with acrolein for 30 min and lysed in RIPA buffer. IKK was immunoprecipitated from cell lysates and derivatized with DNPH, and subsequently analyzed by SDS-PAGE and western blotting using an α-DNP antibody (**left**) or an antibody against IKKβ (**right**). One representative experiment of two is shown. HC, IgG heavy chain.

DISCUSSION

The present study demonstrates the capacity of acrolein to modulate IL-8 expression in tracheobronchial epithelial cells, and provides insights into the molecular mechanism by which acrolein may be causing alterations in inflammatory processes. Central to this antiinflammatory property is the ability of acrolein to inhibit the activation of NFκB, a critical event in airway epithelial cell inflammation (29). The inhibitory effects of acrolein may include direct alkylation of IKK by Michael adduct formation with a reactive cysteine residue in the activator loop of the β-subunit of IKK (16, 31), as indicated by increased DNPH reactivity. In this regard, the inhibitory action of acrolein appears analogous to those of other lipid peroxidation products, such as 4HNE or cyclopentenone prostaglandins, which can inhibit NFκB activation and reduce expression of target genes, including proinflammatory cytokines (15). However, some caution is required as our results do not directly demonstrate alkylation of the active-site cysteine residue in IKKβ, and other (indirect) modifications may have given rise to the observed increase in DNPH reactivity (see below).

Suppression of IL-8 release from HBE1 or NHBE following exposure to acrolein was detected at the protein level, as well as the mRNA level, suggesting that acrolein modulates IL-8 expression at the transcriptional level. As acrolein is a major component of CS, it may affect the activation of inflammatory-immune processes by CS. Several studies have demonstrated proinflammatory effects of CS extracts and some individual CS components *in vitro* using cultured epithelial cells, as well as *in vivo* (1, 12, 24). The inhibitory effects of acrolein described in the present study and in previous studies (15) illustrate the complexity of pro- and antiinflammatory effects by the multitude of bioactive substances present in CS. The production of various cytokine and chemokine mediators is required to allow the coordinated orchestration of immune cell behavior between lung epithelial cells, alveolar macrophages, and infiltrated T cells and granulocytes at the insulted area, with the aim of deactivating and/or removing "foreign" material. Careful regulation of both initiation and resolution of inflammatory responses is a part of this overall process. Such cell-to-cell communication may be impaired by inhaled agents such as acrolein, thereby resulting in disregulation of inflammatory-immune responses.

Our results suggest that acrolein inhibits TNFα-induced cytokine production by inhibiting NFκB activation in human tracheobronchial epithelial cells. NFκB is the primary transcription factor critical for regulating the immune response to many pathogenic signals, and activation of NFκB is required for inflammatory cytokine release by epithelial cells during infection. Similarly, inhibition of NFκB activation may be responsible for acrolein-induced inhibition of macrophage cytokine release (20). Furthermore, it is well known that NFκB is implicated in the gene expression of T and B cells (4, 10); therefore, acrolein inhibition of NFκB activity in B and T cells within the lung may also contribute to acrolein-induced immunosuppression in the lung. The collective effects of acrolein on suppression of cytokine release from a number of immune regulatory cells, in combination with additional effects on other specific cell functions, may contribute to the overall immunoregulatory effects of acrolein exposure.

The present results do not establish the precise mechanism involved in inhibition of NFκB activation and IL-8 expression by acrolein, but they strongly suggest interference with an upstream event involved in NFκB activation, as illustrated by the inhibition of IκBα phosphorylation and degradation and the direct modification of IKKβ. Nevertheless, we cannot exclude the involvement of more direct inhibition of TNFα receptor activation or effects on other upstream signaling processes (11). Like other α,β-unsaturated aldehydes, acrolein reacts with cysteine, histidine, and lysine residue proteins by Michael addition (36). Similar direct modification of IKKβ has been demonstrated by 4HNE (16) and by antiinflammatory cyclopentenone prostaglandins, which inhibit NFκB activation by covalent modification of IKK protein by a direct reaction with a cysteine residue in the activation loop (31). Nevertheless, increased DNPH reactivity cannot be interpreted as direct evidence for alkylation of the active-site cysteine residue in IKKβ, and may also reflect indirect formation of protein carbonyls by secondary oxidative events, although direct alkylation of IKKβ could theoretically be demonstrated with a monoclonal antibody against protein–acrolein adducts that we have used in previous studies (*e.g.,* 26). However, this antibody recognizes a specific adduct with lysine residues, which would be distinct from the anticipated alkylation product with the redox-sensitive cysteine residue in the transactivation loop (Cys^{179}) that is also the putative target for analogous alkylating agents (16, 31). Further studies will be needed to demonstrate more definitively whether and how acrolein interacts with IKK and how this affects its activation.

In addition to direct inactivation of IKK, acrolein could also indirectly modulate inflammatory signaling by decreasing cellular reduced glutathione (GSH) content (25), which could indirectly affect redox-sensitive events in NFκB activation. For instance, the Cys^{62} residue in the p50 subunit and the Cys^{38} residue in the p65 subunit of NFκB are essential for DNA binding and transcriptional activation (2); hence, the lack of reducing conditions within the nuclear compartment can compromise NFκB DNA binding. Indeed, oxidation of cysteines in the DNA-binding domain of NFκB subunit p50 affects NFκB DNA-binding capacity (22). Moreover, ubiquitin-conjugating enzymes are inhibited by high levels of oxidized glutathione (27), which would result in accumulation of phosphorylated IκB without release of active NFκB. Finally, depletion of cellular GSH by acrolein may also favor oxidative processes that may result in oxidative modification of Cys^{179} in IKKα and IKKβ, which results in the inactivation of IKK activity as has been shown for arsenite (24) and hydrogen peroxide (19). To address whether changes in GSH status can indirectly affect IKK activity, inhibition of GSH synthesis with buthionine sulfoximine could be used. Studies in similar epithelial cell systems have shown, however, that such inhibition of GSH synthesis and depletion of cellular GSH do not alone cause inactivation of IKK or NFκB signaling (N. Reynaert and Y. Janssen-Heininger, personal communication). Hence, the suspected inactivation of IKK in the present study is likely a direct result of direct or indirect modification due to acrolein, and not an indirect consequence of depletion of GSH. However, as was observed in a previous similar study (18), acrolein exposure does cause significant depletion of cellular GSH (to ~20–30% of initial levels after treatment with 25 μ*M* acrolein).

In a recent study, Biswal and co-workers have shown inhibition by acrolein of activator protein-1, another redox-sensitive transcription factor, both through the changes in cellular thiol redox balance and by covalent modification of c-jun by acrolein (3). Furthermore, our previous studies have demonstrated that acrolein can interfere with respiratory burst activation and with apoptotic pathways in neutrophils (8). Thus, the cellular effects of acrolein are undoubtedly not specific for IKK or NFκB signaling, but comprise many other potential effects on other signaling or regulator pathways. Overall, the current data support the hypothesis that inhalation of acrolein, as a major component of CS or other forms of environmental pollution, may contribute to alterations in inflammatory-immune regulation and in host defense that are caused by tobacco smoke exposure. The present studies demonstrating reduced IL-8 production by tracheobronchial epithelial cells, in association with inhibition of NFκB activation, may provide another piece of this complex puzzle. The overall significance of these findings to altered inflammation and/or carcinogenesis in association with cigarette smoking will need to be determined in future studies.

ACKNOWLEDGMENTS

This work was supported by grants from NIH (HL68865) and the University of California Tobacco-Related Disease Research Program (7RT-0167).

ABBREVIATIONS

CS, cigarette smoke; Dig, digoxygenin; α-DNP, α-dinitrophenol; DNPH, 2,4-dinitrophenylhydrazine; DTT, dithiothreitol; ECL, enhanced chemiluminescence; ELISA, enzyme-linked immunosorbent assay; EMSA, electrophoretic mobility shift assay; GSH, reduced glutathione; HBE1, immortalized human bronchial epithelial cells; 4HNE, 4-hydroxynonenal; IKK, IκB kinase; IL-8, interleukin-8; NFκB, nuclear factor-κB; NHBE, normal human bronchial epithelial cells; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PMSF, phenylmethylsulfonyl fluoride; RT-PCR, reverse transcription–polymerase chain reaction; SDS, sodium dodecyl sulfate; TNFα, tumor necrosis factor-α.

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Received for publication February 24, 2002; accepted August 23, 2004.

This article has been cited by:

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