Effect of acrolein on human alveolar macrophage NF-κB activity

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Effect of acrolein on human alveolar macrophage NF-κB activity. Am. J. Physiol. 277 (Lung Cell. Mol. Physiol. 21): L550–L557, 1999.—Acrolein is an environmental pollutant that is known to suppress respiratory host defense against infections; however, the mechanism of the decrease in host defense is not yet clear. We have previously reported that acrolein inhibited endotoxin-induced cytokine release and induced apoptosis in human alveolar macrophages, suggesting that the inhibition of cytokine release and/or cytotoxicity to alveolar macrophages may, in part, be responsible for acrolein-induced immunosuppression in the lung. Because nuclear factor-κB (NF-κB) is an important transcription factor for a number of cytokine genes and is also an important regulator of apoptosis, the effect of acrolein on NF-κB activity was examined by electrophoresis mobility shift assay. Acrolein caused a dose-dependent inhibition of endotoxin-induced NF-κB activation as well as an inhibition of basal level NF-κB activity. Because IκB is a principal regulator of NF-κB activity in the nucleus, changes in IκB were determined by Western blotting. Acrolein-inhibited IκB phosphorylation leads to an increase in cellular IκB levels preventing NF-κB nuclear translocation and is likely the mechanism of acrolein-induced inhibition of NF-κB activity. The role of basal level NF-κB in acrolein-induced apoptosis was also examined. An NF-κB inhibitor (MG-132) also induced apoptosis in human alveolar macrophages, suggesting that a certain basal level NF-κB activity may be required for macrophage cell survival. Taken together, our results suggest that the acrolein-inhibited endotoxin-induced NF-κB activation decreased the basal level NF-κB activity, which may be responsible for the inhibition of cytokine release and the induction of apoptosis in human alveolar macrophages.

nuclear factor-κB; IκB; apoptosis; cytokine; MG-132

ACROLEIN (CH₂CHCHO) is a volatile, highly inflammatory liquid with a pungent, lacrimary, and irritating odor. It is an air pollutant generated by incomplete combustion or pyrolysis of organic materials such as fuels, wood, synthetic polymers, food, and tobacco (3, 39). Because of the source distribution, the general population is exposed to acrolein mainly in indoor and, to a lesser extent, outdoor air. However, acrolein has been identified as the noncancer hazardous air pollutant of greatest health concern (11).

Acrolein has been reported to be a highly selective toxin of the respiratory tract for humans and experimental animals (1, 39). Acute inhalation of acrolein vapor has been reported to cause degeneration of the respiratory epithelium in rats, hamsters, and rabbits (39). In addition, short-term acrolein inhalation exposure decreased bactericidal activity within the respiratory tract in experimental animals (2, 3). Acrolein has also been proposed to contribute to deficiencies in lung host defense against nonspecific respiratory infections in cigarette smokers (15, 16, 21). However, only limited information is available on the mechanism of acrolein-induced injury, especially on decreased microbicidal activity and immunosuppression in the lung.

Alveolar macrophages (AMs) are the key lung cells involved in nonspecific host defense and are considered to play a central role in the regulation of the immune response to inhaled pathogens and development of inflammation (14, 20). The adverse effects of acrolein on AMs have been studied in a number of animal models and include inhibition of macromolecule synthesis (23), ATPase activity, and phagocytosis (27). In addition, acrolein has been reported to modulate arachidonic acid metabolism (18) and change phagocytic and enzymatic patterns (33) in macrophages. Previously, Li et al. (25) reported that acrolein caused a dose-dependent inhibition in the release of interleukin (IL)-1β, IL-12, and tumor necrosis factor (TNF)-α in vitro from endotoxin-stimulated human AMs. Acrolein also caused a dose-dependent induction of a stress response, apoptosis, and necrosis in human AMs (25). It is possible that the inhibition of cytokine release and cytotoxicity to AMs by acrolein may contribute to the immunosuppression in the lung by acrolein. However, the molecular mechanism of acrolein-induced inhibition in cytokine release and induction of apoptosis is yet not clear.

In general, macrophage cytokine release is predominantly regulated by the transcription rates of cytokine genes. Nuclear factor-κB (NF-κB) is a primary transcription factor for inflammatory cytokines such as IL-1β, IL-12, and TNF-α (9). NF-κB is known to be activated by a wide range of stimuli, including bacterial endotoxin, mitogens, viral proteins, ionizing radiation, and ultraviolet radiation (5, 34). NF-κB activity is negatively regulated by a family of inhibitor proteins known as IκB. Activation of NF-κB requires that IκB be phosphorylated, allowing the NF-κB dimer to dissociate from IκB. Dissociated NF-κB can translocate into the cell nucleus, bind to its target DNA sequence, and activate the transcription of its target genes (9, 36). After phosphorylation of IκB, it is ubiquitinated and degraded (9, 36).

In addition to being an important transcription factor for a number of cytokine genes, NF-κB is also important for cell survival. Mice lacking Rel A [Rel A(−/−); p65 subunit of NF-κB] died at the embryonic stage due to extensive apoptosis in the liver (8). Fibroblasts and...
macrophages from Rel A(−/−) mice are sensitive to TNF-α-induced cell death, and reintroduction of Rel A to Rel A(−/−) fibroblasts enhanced cell survival (7). Furthermore, a study (38) has shown that the activation of NF-κB protected cells from apoptosis induced by TNF-α and ionizing radiation, whereas the inhibition of NF-κB activity increased sensitivity to TNF-α-induced apoptosis (37, 38).

The important roles of NF-κB in regulating inflammatory cytokine gene expression and in protecting cells from apoptosis makes it a logical target to examine acrolein-induced inhibition of cytokine release and induction of apoptosis. Therefore, in this study, the effects of acrolein on the regulation of NF-κB activity and the role of NF-κB on cell survival were examined in human AMs in vitro.

METHODS

Materials. Acrolein and fetal bovine serum (FBS) were obtained from Sigma (St. Louis, MO). Medium 199 was obtained from Gibco BRL (Life Technologies, Gaithersburg, MD). DNA oligonucleotides for the NF-κB consensus sequence 5'-AGT TGA GGG GAC TTT CCC AGG C-3' and antibodies against IκB-α and phosphorylated IκB-α were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against actin, horseradish peroxidase-linked anti-rabbit Ig whole antibody (from donkey), and horseradish peroxidase-linked anti-mouse Ig whole antibody (from sheep) were obtained from Amersham (Arlington Heights, IL).

Isolation and culture of human AMs. Cells were obtained by bronchoalveolar lavage of healthy volunteers by methods described elsewhere (13, 19, 31). In brief, instillations of 140 ml of sterile saline resulted in recoveries of 80–100 ml of lavage fluid that was kept at 4°C until the cells were isolated from the lavage fluid by a 4°C centrifugation at 500 g. The cell pellet was resuspended in a small volume of Medium 199 containing 10% heat-inactivated FBS and added antibiotics (50 U/ml of penicillin, 50 µg/ml of gentamicin, and 50 µg/ml of BRL) with 10% heat-inactivated FBS and added antibiotics. The cell suspension was then lysed in a glass Dounce homogenizer (Wheaton, Millville, NJ) with 20 strokes of an A-type pestle. The nuclei were placed into 1.5-ml Eppendorf tubes, pelleted at 12,000 × g for 10 min, and extracted with 50 µl of modified Dignam et al. solution C (100 mM HEPES, 25% glycerol, 1 mM leupeptin, and 400 mM NaCl, pH 8.0). The nuclear extracts were obtained after centrifugation at 25,000 × g for 10 min. Protein concentrations were determined with Bio-Rad protein assay dye reagent, with bovine serum albumin as the standard.

NF-κB activity in the nuclear proteins were analyzed by electrophoretic mobility shift assay as described by Misra et al. (28a). Oligonucleotides for NF-κB binding sequence were 5'-end labeled with [γ-32P]ATP by T4 polynucleotide kinase (Promega, Madison, WI). Each sample (20 µl) had 5 µg of nuclear extract, 0.1 ng of 32P-labeled DNA, 1 µg of poly(dI-dC), 100 mM NaCl, 25 mM HEPES, 6.25% glycerol, and 0.25 mM leupeptin, pH 8.0. The reaction mixtures were loaded onto 6% polyacrylamide gels in 0.25 µM dGTP, 200 µM dTTP, 2 µl of [γ-32P]dCTP by T4 polynucleotide kinase (Promega, Madison, WI). Each sample (20 µl) had 5 µg of nuclear extract, 0.1 ng of 32P-labeled DNA, 1 µg of poly(dI-dC), 100 mM NaCl, 25 mM HEPES, 6.25% glycerol, and 0.25 mM leupeptin, pH 8.0. The reaction mixtures were loaded onto 6% polyacrylamide gels in 0.25x buffer consisting of 22 mM Tris, 22 mM sodium borate, and 0.5 mM EDTA, pH 8.0. After electrophoresis at 10 V/cm, the gels were dried at 70°C under vacuum in a gel dryer and then exposed to X-ray film.

To visualize DNA fragmentation, genomic DNA was isolated from treated cells with the DNA ISOLATOR (Genosys, The Woodlands, TX) and 3'-end labeled with [α-32P]dCTP (ICN) by incubation of 1 µg of DNA in 50 µl of reaction buffer (50 mM Tris-HCl, pH 7.6, 10 mM MgCl2, 200 µM dATP, 200 µM dGTP, 200 µM dTTP, 2 µl of [α-32P]dCTP, and 2 U of Klenow) at 37°C for 30 min. The same amount of [α-32P]dCTP-labeled DNA (50 ng) for each sample was loaded onto a 2% agarose gel and run at 5 V/cm for 5 h in 40 mM Tris-acetate buffered ionic strength (12% Ready Gels, Bio-Rad, Hercules, CA). Total proteins from an equal number of cells (2 × 106 cells/sample) were loaded onto each lane. Resolved proteins were transferred to nitrocellulose membranes (Amersham) with a Trans-Blot Electrophoretic Transfer Cell (Bio-Rad). The membranes were incubated for 16 h at 4°C in blocking buffer (5% Blotto in 10 mM Tris-HCl, pH 7.2, and 150 mM NaCl) and were then incubated with primary antibody (1:200 for IκB-α, 1:1,000 for phosphorylated IκB-α, and 1:2,000 for actin) for 16 h at 4°C. The membranes were washed extensively with 10 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween, pH 8.0, incubated with the secondary antibody conjugated to horseradish peroxidase at a 1:10,000 dilution for 1 h at room temperature, and washed extensively with 10 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween. Enhanced chemiluminescence detection (Amersham) followed by autoradiography (Hyperfilm-ECL, Amersham) was used to visualize the proteins.

Electrophoretic mobility shift assay. Nuclear extracts from AMs were prepared by a modification of the procedure of Misra et al. (28a). In brief, 2.5 × 106 cells were washed twice with 3 ml of PBS, washed once with 3 ml of modified Dignam et al. solution C (2.5 mM MgCl2 and 10 mM HEPES, pH 8.0), and suspended in 1 ml of solution A. The cell suspensions were then placed into 1.5-ml Eppendorf tubes, pelleted at 12,000 × g for 10 min, and extracted with 50 µl of modified Dignam et al. solution C (100 mM HEPES, 25% glycerol, 1 mM leupeptin, and 400 mM NaCl, pH 8.0). The nuclear extracts were obtained with centrifugation at 25,000 × g for 10 min. Protein concentrations were determined with Bio-Rad protein assay dye reagent, with bovine serum albumin as the standard.

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To visualize DNA fragmentation, genomic DNA was isolated from treated cells with the DNA ISOLATOR (Genosys, The Woodlands, TX) and 3'-end labeled with [α-32P]dCTP (ICN) by incubation of 1 µg of DNA in 50 µl of reaction buffer (50 mM Tris-HCl, pH 7.6, 10 mM MgCl2, 200 µM dATP, 200 µM dGTP, 200 µM dTTP, 2 µl of [α-32P]dCTP, and 2 U of Klenow) at 37°C for 30 min. The same amount of [α-32P]dCTP-labeled DNA (50 ng) for each sample was loaded onto a 2% agarose gel and run at 5 V/cm for 5 h in 40 mM Tris-acetate buffered ionic strength (12% Ready Gels, Bio-Rad, Hercules, CA). Total proteins from an equal number of cells (2 × 106 cells/sample) were loaded onto each lane. Resolved proteins were transferred to nitrocellulose membranes (Amersham) with a Trans-Blot Electrophoretic Transfer Cell (Bio-Rad). The membranes were incubated for 16 h at 4°C in blocking buffer (5% Blotto in 10 mM Tris-HCl, pH 7.2, and 150 mM NaCl) and were then incubated with primary antibody (1:200 for IκB-α, 1:1,000 for phosphorylated IκB-α, and 1:2,000 for actin) for 16 h at 4°C. The membranes were washed extensively with 10 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween, pH 8.0, incubated with the secondary antibody conjugated to horseradish peroxidase at a 1:10,000 dilution for 1 h at room temperature, and washed extensively with 10 mM Tris-HCl, 150 mM NaCl, and 0.05% Tween. Enhanced chemiluminescence detection (Amersham) followed by autoradiography (Hyperfilm-ECL, Amersham) was used to visualize the proteins.
buffer, pH 8.0, with 1 mM EDTA. The gel was dried at 80°C under vacuum in a gel dryer and then exposed to X-ray film.

**Statistical analysis.** Values are presented as means ± SE. Statistical differences between control and treated groups were determined by a one-way analysis of variance followed by Student-Newman-Keuls test. Differences were considered significant at $P < 0.05$.

**RESULTS**

Effects of acrolein on lipopolysaccharide-induced NF-κB activity. Li et al. (25) recently reported that acrolein inhibited endotoxin-induced cytokine release in human AMs. Because NF-κB is the critical transcription factor for many inflammatory cytokines, the effects of acrolein on endotoxin-induced NF-κB activity was examined by electrophoretic mobility shift assay. As shown in Fig. 1, endotoxin treatment caused an activation of NF-κB activity and acrolein caused a dose-dependent inhibition of endotoxin-induced NF-κB activation in human AMs. A 25 µM dose of acrolein completely blocked endotoxin induction of NF-κB activity. Acrolein treatment (50 µM for 4 h) also caused a decrease in the basal level NF-κB activity in unstimulated macrophages (Fig. 1). The specificity of NF-κB binding was confirmed by competition and supershift assay (data not shown). Because NF-κB is the critical transcription factor that regulates the expression of many inflammatory cytokine genes, it is likely that the inhibition of endotoxin-induced NF-κB activation by acrolein may contribute to acrolein-induced inhibition of cytokine release.

**Effects of acrolein on basal level NF-κB activity.** The dose response and time course for the effect of acrolein on basal (unstimulated) level NF-κB activity in human AMs were also examined by electrophoretic mobility shift assay as described in METHODS. Acrolein treatment for 6 (A) and 24 (B) h. NF-κB activity (n = 3 nuclear extracts) was examined by electrophoretic mobility shift assay as described in METHODS. Acrol 10 µM, cells treated with 10 µM acrolein.

**Effect of acrolein on cellular IκB-α level.** Considering that NF-κB activity is regulated by IκBs and the best-characterized IκB is IκB-α (9, 36), the effect of
Acrolein on the basal levels of IκB-α in human AMs was examined by Western blotting. As shown in Fig. 3, acrolein caused a dose-dependent increase in IκB-α levels within 1 h. Although no apparent change in the level of IκB-α was evident at 10 µM, large increases in IκB-α were evident at 25 µM and higher doses of acrolein. Based on these findings, it is likely that the increase in cellular IκB-α levels may be responsible for the inhibition of NF-κB activity by acrolein.

Effect of acrolein on IκB-α phosphorylation. The increase in cellular IκB-α levels could be caused by an increase in IκB-α synthesis and/or a decrease in IκB-α degradation. Experiments with an inhibitor of protein synthesis indicated that an acrolein-induced increase in IκB-α is independent of protein synthesis (data not shown), suggesting that the decrease in IκB-α degradation is responsible for the increase in IκB-α. Degradation in IκB is regulated by phosphorylation: IκBs are first phosphorylated, then ubiquitinated and degraded during NF-κB activation (4, 6, 36). Therefore, the effect of acrolein on IκB-α phosphorylation was examined. As shown in Fig. 4, acrolein caused a dose-dependent decrease in phosphorylated IκB-α in human AMs within 1 h. Decreased phosphorylation was evident with even the lowest dose of acrolein examined (10 µM). This result suggests that the decrease in phosphorylated IκB-α levels in the cells is likely responsible for acrolein-induced decreases in IκB-α degradation and increases in cellular IκB-α levels in human AMs.

Effects of NF-κB inhibition on AM cell survival. Inhibition of NF-κB activity has been demonstrated to cause increased sensitivity to TNF-α-induced apoptosis (37, 38), suggesting that the level of NF-κB is important for cell survival. Our results demonstrate that acrolein decreased basal level NF-κB activity in human AMs (Fig. 2) and, at similar concentrations, caused apoptosis in human AMs (25). However, it is not known whether the inhibition of basal level NF-κB activity is responsible for acrolein-induced apoptosis. Therefore, the effects of NF-κB inhibition on human AM cell survival were examined with NF-κB inhibitors. SN-50 has been reported to inhibit NF-κB nuclear translocation in endothelial cells (26). MG-132 (Z-Leu-Leu-Leu-H) is a proteasome inhibitor reported to inhibit NF-κB activity. 

Fig. 3. Effect of acrolein on cellular IκB-α levels. Human alveolar macrophages were incubated with different concentrations of acrolein for 1 h, and cellular IκB-α levels (n = 4 nuclear extracts) were examined by Western blotting as described in METHODS. Actin serves as a marker for equal protein loading in each lane.

Fig. 4. Effect of acrolein on IκB-α phosphorylation. Human alveolar macrophages were incubated with different concentrations of acrolein for 1 h, and cellular phosphorylated (P) IκB-α levels (n = 4 nuclear extracts) were examined by Western blotting as described in METHODS. Actin serves as a marker for equal protein loading in each lane.

Fig. 5. Effects of NF-κB inhibitors on alveolar macrophage NF-κB activity. Human alveolar macrophages were incubated with inhibitors for 24 h, and NF-κB activity was measured by electrophoretic mobility shift assay as described in METHODS. SN-50, cells treated with SN-50 (20 µM); MG-132, cells treated with MG-132 (5 µM).
NF-κB activity in a number of cell types (12, 30). The efficacy of these inhibitors on human AM NF-κB activity was tested by electrophoretic mobility shift assay (Fig. 5). Twenty-four hours after treatment, MG-132 (5 µM) completely blocked NF-κB activity, whereas SN-50 (20 µM) had no effect on NF-κB activity in human AMs.

The possibility of induction of apoptosis by NF-κB inhibition was examined by Cell Death ELISA, a procedure that measures cytosolic DNA fragmentation during apoptosis (24). MG-132 caused a significant increase in cytosolic DNA fragments (Fig. 6), suggesting apoptosis in human AMs. In contrast, SN-50 had no effect on cytosolic DNA fragmentation, i.e., no apoptosis in treated cells. The induction of apoptosis by MG-132 was confirmed by morphological examination (Fig. 7).

Fig. 6. Effects of NF-κB inhibitors on cytosolic DNA fragmentation. Human alveolar macrophages were treated with acrolein and NF-κB inhibitors for 24 h. Cytosolic DNA fragmentation was examined by Cell Death ELISA as described in METHODS. Cells (1 × 10⁵) from each condition were processed, 5,000 cells were used for each reaction, and triplicate reactions were performed for each condition. Results are optical density at wavelength of 405 nm from at least 6 separate experiments. *Significant difference between treatment and control groups, P < 0.05.

MG-132, as well as acrolein, caused morphological changes in human AMs, such as shrinkage of the cytoplasm, nuclear condensation, and fragmentation, that were characteristic of apoptosis. The apoptotic DNA fragmentation induced by MG-132 and acrolein was confirmed by DNA gel electrophoresis. Both MG-132 and acrolein caused internucleosomal DNA fragmentation, resulting in a DNA ladder of multiples of 180–200 bp (Fig. 8). The formation of internucleosomal fragments in DNA from MG-132- and acrolein-treated cells confirmed that cells were dying by an apoptotic mechanism. The induction of apoptosis by the NF-κB inhibitor MG-132 suggests that basal level NF-κB activity may be required for human AM cell survival.

DISCUSSION

In the present study, we provide new information on the molecular mechanism by which acrolein may be causing alterations in host defense and apoptosis. Central to both effects is the ability of acrolein to block the nuclear activity of NF-κB. This study, therefore, not only provides new information on the molecular targets of acrolein in human cells, which would be exposed to acrolein in the ambient environment or through cigarette smoke, but also confirms the importance of NF-κB in macrophage cytokine release. In addition, the work provides evidence for the important role of NF-κB in regulating apoptosis of human AMs.

We demonstrated that acrolein inhibits endotoxin-induced NF-κB activation in human AMs. NF-κB is a primary transcription factor critical for regulating the immune response to many pathogenic signals, and activation of NF-κB is required for inflammatory cytokine release by AMs during infection (5, 29). Consequently, inhibition of endotoxin-induced activation of NF-κB may be responsible for acrolein-induced inhibition of macrophage cytokine release (25). Also, it is well known that NF-κB plays a central role in the regulation

Fig. 7. Morphology of human alveolar macrophages treated with acrolein and NF-κB inhibitors. Macrophage apoptosis induced by acrolein was examined by cell staining followed by light microscopy as described in METHODS. A: control cells. B: cells treated with acrolein (25 µM). C: cells treated with MG-132 (5 µM). D: cells treated with SN-50 (20 µM). Arrowheads, apoptotic cells.
It has been reported that endotoxin-stimulated release of TNF-α, IL-1β, and IL-12 from human AMs had different sensitivities to inhibition by acrolein, with TNF-α release being the least-sensitive response (25). Endotoxin-stimulated TNF-α release has been reported to involve both transcriptional and translational activation (22). On endotoxin stimulation, TNF-α transcription is increased 5- to 50-fold, whereas its translation was increased >100-fold. This could account for the decreased sensitivity of endotoxin-induced TNF-α release to acrolein treatment compared with that of IL-1β and IL-12. Also, the regulation of different cytokine gene transcription involves NF-κB as well as other transcription factors such as Sp1, Ets, ATF-2/c-Jun, and NF-IL-6 (36). Consequently, the interaction of NF-κB and other transcription factors will determine the final rates of transcription of specific cytokines. Therefore, the difference in acrolein sensitivity of endotoxin-induced cytokine release could be accounted for by other transcription factors working with NF-κB that may or may not be affected by acrolein treatment.

NF-κB has been reported to protect cells against apoptosis induced by TNF-α, ionizing radiation, and the chemotherapeutic compound daunorubicin (38). Correspondingly, inhibition of NF-κB activity increased the sensitivity to TNF-α-induced apoptosis in a number of cell types (7, 37, 38). In our study, we demonstrated that acrolein decreased the basal level NF-κB activity in human AMs. The relationship between a decrease in the basal level NF-κB activity to acrolein-induced apoptosis cannot be established equivocally from these results. However, it suggests that the inhibition of basal level NF-κB activity may be responsible for acrolein-induced apoptosis because the NF-κB inhibitor MG-132 also induced apoptosis in human AMs.

It is likely that acrolein and MG-132 inhibit NF-κB activity by different mechanisms. We propose, as described below, that an acrolein-induced decrease in NF-κB activity is through the blocking of IkB-α phosphorylation. In contrast, it has been reported that MG-132 decreases NF-κB by blocking proteasome degradation of IkB and increasing levels of phosphorylated IkB-α (12, 30). Nevertheless, the fact that both acrolein and MG-132 caused apoptosis suggests that basal level NF-κB activity may be important (or required) for AM cell survival.

The possibility that basal level NF-κB activity is required for cell survival is intriguing, even though previous studies (8, 32) on the role of basal level NF-κB activity on cell survival have not provided compelling evidence. It has been reported that Rel A (−/−) cells or cells lacking p50 are viable in culture (8, 32). However, because NF-κB is a family of dimeric transcription factors (4, 36), the full impact of deficiencies of one Rel protein on cell survival may be masked by the redundancy of the NF-κB/Rel protein family. It is worth noting that mice lacking Rel A have excessive apoptosis in the liver (8). Cell lines transfected with dominant negative IkB-α (IkB-αM) or treated with MG-132 were also viable in culture (37, 38). It is possible that permanently differentiated cells such as AMs are more...
sensitive to the effects of NF-κB inhibition. Further studies will be needed to elucidate the role of basal level NF-κB activity on AM cell survival.

The present study provides a mechanism to explain decreased NF-κB activity by acrolein. The regulation of NF-κB activity is centered on phosphorylation, ubiquitination, and/or degradation of IκB (4, 6, 28, 35, 36). The phosphorylation of IκB is catalyzed by the IκB kinase (IKK) complex. For example, the signal for receptor-mediated NF-κB activation is transduced by the TNF receptor-associated factor (TRAF) family of adapter proteins such as TRAF2 and TRAF6. The resulting activation of mitogen-activated protein kinase kinases such as NF-κB-inducing kinase and mitogen-activated protein/extracellular signal-regulated kinase kinase-1, by TRAF leads to the phosphorylation of IκKα and IκKβ and the activation of IKK. The activation of IKK leads to the phosphorylation of IκB, which signals the ubiquitination and degradation of IκB. The IκB-free NF-κB is translocated into the nucleus where it activates the transcription of its target genes (28, 35). Our study suggests that the inhibition of NF-κB activity by acrolein is associated with a decrease in IκB-α phosphorylation, resulting in an increase in IκB-α levels. It is possible (yet to be confirmed) that acrolein has similar effects on other members of the IκB family. We propose that the decrease in IκB phosphorylation and increase in IκB levels are likely the cause of acrolein-induced inhibition of NF-κB activity. However, it is not clear if the inhibition of IκB phosphorylation by acrolein is through direct inhibition of IKK or through inhibition of upstream regulators of the NF-κB activation pathway.

In summary, our results demonstrate that acrolein caused a dose-dependent inhibition of endotoxin-induced NF-κB activity. The inhibition of endotoxin-induced NF-κB activity is likely responsible for acrolein-induced inhibition of endotoxin-induced cytokine release. Also, our results demonstrate that acrolein caused an inhibition of basal level NF-κB activity, and the inhibition of IκB phosphorylation is likely the mechanism of acrolein-induced decrease in NF-κB activity. Furthermore, the inhibition of basal level NF-κB activity may be responsible for acrolein-induced apoptosis. The requirement of basal level NF-κB activity for human AMs may have implications on other cell types.

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