Reactive oxygen intermediates stimulate interleukin-6 production in human bronchial epithelial cells

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Yoshida, Yoshimasa, Muneharu Maruyama, Tadashi Fujita, Nobuki Arai, Ryuji Hayashi, J un Araya, Shoko Matsu, Naohiro Yamashita, Eiji Sugiyama, and Masa- shi Kobayashi. Reactive oxygen intermediates stimulate interleukin-6 production in human bronchial epithelial cells. Am. J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L900–L908, 1999.—Reactive oxygen intermediates (ROIs) play an important role in the initiation and progression of lung diseases. In this study, we investigated whether ROIs were involved in the induction of interleukin (IL)-6 in human bronchial epithelial cells. We exposed normal human bronchial epithelial cells as well as a human bronchial epithelial cell line, HS-24, to ROIs. We measured the amount of IL-6 in the culture supernatants using ELISA and the IL-6 mRNA levels using RT-PCR. Superoxide anions (O_2^-), but not hydrogen peroxide (H_2O_2), increased IL-6 production. To examine whether it is a cell type-specific mechanism of airway epithelial cells, the experiments were also performed in human lung fibroblasts, WI-38-40. In WI-38-40 cells, neither O_2^- nor H_2O_2 increased IL-6 production. In contrast, tumor necrosis factor (TNF)-a (200 U/ml) induced IL-6 at the protein and mRNA levels in both airway epithelial cells and lung fibroblasts. This cytokine-induced IL-6 production was significantly suppressed by several antioxidants, including dimethyl sulfoxide (DMSO), in airway epithelial cells. In WI-38-40 cells, DMSO was not able to suppress IL-6 production induced by TNF-a. Pretreatment with DMSO recovered the TNF-a-induced depletion of intracellular reduced glutathione in HS-24 cells. These findings indicate that oxidant stress specifically induces IL-6 production in human bronchial epithelial cells and that in these cells ROIs may be involved in IL-6 production after stimulation with cytokines such as TNF-a. Presumably, ROIs participate in the local immune response in lung diseases via IL-6 release from bronchial epithelial cells.

airway epithelial cells; interleukin-6; reactive oxygen intermediates; reduced glutathione; superoxide anions

NUMEROUS STUDIES (15, 30) have revealed that reactive oxygen intermediates (ROIs) play a crucial role in the initiation and progression of a wide range of diseases and in the regulation of a number of important biological processes. The lung is a major target organ for oxidant injury because ROIs are generated by inflammatory cells and by chemotherapeutic agents that concentrate in the lung. Furthermore, various forms of lung diseases require O_2^- therapy, which adds to the oxidant burden on the lung (15, 16, 30). In the lung, ROIs can induce a variety of lesions in the respiratory tract as well as in the pulmonary vasa vasculare and parenchyma.

It has been reported that bronchial epithelial cells release soluble mediators on exposure to ROIs (2, 11). Adler et al. (2) have demonstrated that ROIs stimulate release of high-molecular-weight glycoconjugates from rodent respiratory epithelial cells in vitro. DeForge et al. (11) have reported that ROIs induce the production of interleukin (IL)-8 in A549 human type II pulmonary epithelial cells. There is also growing evidence that ROIs can be released by many types of cells in response to a variety of stimuli, such as tumor necrosis factor (TNF)-a and lipopolysaccharide (3, 14, 23, 24), and that ROIs can serve as intracellular signals for gene activation involving specific transcription factors such as nuclear factor (NF)-a (31, 32).

IL-6 is a cytokine produced by a variety of cells including human bronchial epithelial cells (8, 35). IL-6 has multiple biological activities, which include proliferation of hemopoietic stem cells, potentiation of T-cell proliferative responses, and induction of hepatic acute-phase protein synthesis (4). Several experimental studies (8, 35) have demonstrated that the production of IL-6 in airway epithelial cells is modulated by various inflammatory stimuli such as TNF-a. Recently, Simenova et al. (33) have reported that asbestos induces IL-6 in human bronchial epithelial cells via oxidative stress. Despite these studies, there has been no detailed study on IL-6 release in response to ROIs in human airway epithelial cells, including normal human bronchial epithelial (NHBE) cells.

In the present investigation, using NHBE cells as well as a human bronchial epithelial cell line, HS-24, we studied the effects of ROIs on IL-6 release. We also examined whether antioxidants might modulate IL-6 production in these cells stimulated with cytokines such as TNF-a. Finally, we tested whether ROIs were cell type-specific stimuli for IL-6 production in airway epithelial cells.

MATERIALS AND METHODS

Reagents. Xanthine (X), xanthine oxidase (XO), ferricytochrome c, superoxide dismutase (SOD), and 1,3-dimethyl-2-thiourea (DMTU) were purchased from Sigma (Tokyo, J apan). 4'-Hydroxy-3'-methoxyacetophenone was purchased from Aldrich (Tokyo, J apan). Dimethyl sulfoxide (DMSO) was purchased from Wako Pure Chemical Industries (Osaka, J apan). Hydrogen peroxide (H_2O_2) was purchased from Santoku Chemical Industries (Tokyo, J apan). TNF-a was a gift

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from Dainippon Pharmaceutical (Osaka, Japan). IL-1β was a gift from Otsuka Pharmaceutical (Tokyo, Japan).

Cell culture. Cryopreserved primary NHBE cells were purchased from Clonetics (San Diego, CA) and grown in 100-mm tissue culture dishes in bronchial epithelial cell growth medium supplied by Clonetics. The cultures were incubated at 37°C in a humidified, 95% air-5% CO₂ atmosphere. After trypsinization, the cells were subcultured in 24-well culture plates (Corning, New York, NY) at a seeding density of 0.5 × 10⁵ cells/well. When the cells were 50–60% confluent, the medium was changed to bronchial epithelial cell growth medium without hydrocortisone, and the cells were allowed to grow for an additional 24 h. NHBE cells were used within the first five passages.

HS-24 cells, a tumor cell line with properties of human bronchial epithelial cells, were generously provided by Dr. Thomas Muley (Thoraxklinikum, Heidelberg, Germany) (6, 28, 29). This cell line has been used as a model of human airway epithelial cells in the literature (1, 22, 25). WI-38-40 cells were used within the first six passages. WI-38-40 cells were generously provided by Dr. Thomas Muley (Thoraxklinikum, Heidelberg, Germany) (6, 18). Maximum releasable LDH was assessed by incubating cells with 0.2% Tween 20, and background release of LDH was evaluated by incubating cells in medium alone. At the end of the incubation period, LDH release in the supernatant was quantitated. The percentage of cytotoxicity was calculated as follows: [(A – B) / (C – B)] × 100, where A is LDH (in optical density) released into the medium of the test sample, B is LDH released from control cells (i.e., background release), and C is LDH released from cells treated with 0.2% Tween 20.

Statistical analysis. We repeated each type of experiment at least three times and confirmed that similar data were obtained. The results, obtained in triplicate, are presented as means ± SD, and comparisons were made with one-way ANOVA with Fisher’s post hoc test. A P value of < 0.05 was considered statistically significant.

Lactate dehydrogenase cytotoxicity assay. Lactate dehydrogenase (LDH) release from the cells was determined colorimetrically with an LDH assay kit (LDH-Cytotoxic Test Wako, Wako Pure Chemical Industries, Osaka, Japan) (10, 18).

Colorimetric assay for reduced glutathione. Intracellular reduced glutathione (GSH) content was determined colorimetrically with a commercially available kit (BIOXYTECH GSH-400, OXIS International, Portland, OR) (5).

Measurement of superoxide anions. The production of superoxide anions (O₂⁻) by X plus XO was measured as the reduction in ferricytochrome c with the method described by Pick and Mizel (26). X (0.7 mM) and various concentrations of XO (0–20 mU/ml) were incubated with 100 µl of reaction solution containing 160 µM ferricytochrome c in Hank’s balanced salt solution without phenol red at pH 7.4. Then the amount of O₂⁻ generated by reaction of xanthine (X) with xanthine oxidase (XO) was measured as the reduction in absorbance at 550 nm with an iEMS Reader MF microplate reader.

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RESULTS

O2 enhances IL-6 production in bronchial epithelial cells. The initial experiments were performed to determine whether ROIs were capable of inducing IL-6 production in human bronchial epithelial cells. For these experiments, NHBE and HS-24 cells were incubated in the presence of an O2-generating system consisting of X (0.7 mM) and increasing amounts of XO. The amount of O2 generated by this reaction was dependent on the concentration of XO, and the release of O2 was ~10 nmol·ml−1·min−1 at 20 mU/ml of XO (Fig. 1). Alternatively, these cells were exposed to H2O2. XO induced a dose-dependent release of IL-6 in NHBE and HS-24 cells (Fig. 2, A and C). An XO enzyme activity of 5.0 mU/ml led to a twofold increase in IL-6 secretion in NHBE cells. And incubation of HS-24 cells with XO (20 mU/ml) induced a 1.8-fold increase in IL-6 production. Percent cytotoxicity measured by an LDH cytotoxicity assay was 2.8 ± 1.0% in XO (5.0 mU/ml)-exposed NHBE cells and 1.2 ± 1.0% in XO (20 mU/ml)-exposed HS-24 cells. Higher concentrations of XO were not tested because they may cause cell damage as manifested by morphological changes of the monolayer or cell detachment (data not shown) and by the LDH cytotoxicity assay. In contrast, neither cell increased IL-6 production on exposure to H2O2 (Fig. 2, B and D). Incubation of HS-24 cells in the presence of the H2O2-generating enzyme glucose oxidase did not modulate

Fig. 2. Effect of reactive oxygen intermediates (ROIs) on interleukin (IL)-6 production in normal human bronchial epithelial (NHBE) and HS-24 cells. NHBE (A and B) and HS-24 (C and D) cells were incubated for 24 h with XO plus 0.7 mM X (A and C) or H2O2 (B and D). Supernatants were harvested and analyzed for IL-6 by ELISA. Results are means ± SD. Significant difference from unstimulated control sample: *P < 0.05; **P < 0.005; ***P < 0.0001.
the release of IL-6 protein (data not shown). Next, the
time-dependent secretion of IL-6 protein was deter-
mined in HS-24 cells after stimulation with XO (20 mU/ml). As shown in Fig. 3A, stimulation with XO
resulted in a significant increase in the levels of IL-6 at
12 and 24 h compared with those with 0.7 mM X alone
\( (P, 0.0001) \). B: HS-24 cells were incubated in presence of 20 mM XO plus 0.7 mM X. Cellular RNA was harvested at indicated time points and analyzed for IL-6 mRNA expression by RT-PCR. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Antioxidants suppress IL-6 production in bronchial epithelial cells. TNF-\( \alpha \) was dose dependently capable of
inducing IL-6 release from NHBE and HS-24 cells (Fig.
5, A and C). To determine whether ROIs were involved
in IL-6 production in bronchial epithelial cells stimu-
lated with TNF-\( \alpha \), NHBE and HS-24 cells were incubated for 24 h with TNF-\( \alpha \) (200 U/ml) in the presence of various concentrations of an antioxidant, DMSO. DMSO, a known hydroxyl radical (-OH) scavenger, has been found to have beneficial effects in a variety of disease models and can easily penetrate through the cell membranes (38). Measurement of IL-6 levels in the supernatants demonstrated that TNF-\( \alpha \) (200 U/ml) caused a markedly enhanced production of IL-6, and increasing DMSO concentrations resulted in an essentially linear dose-dependent suppression of TNF-\( \alpha \)-induced IL-6 production (Fig. 5, B and D). DMSO was also able to suppress the IL-1\( \beta \)-induced increase in IL-6 release from HS-24 cells after a 24-h incubation (\( P < 0.0001 \); data not shown). In accordance with these protein data, TNF-\( \alpha \) (200 U/ml) significantly increased the levels of IL-6 mRNA in HS-24 cells and DMSO (1.0%) markedly reduced those induced with the cytokine (Fig. 5E). In
contrast, TNF-α and DMSO did not change the levels of GAPDH mRNA. These results agree with the previous reports (3, 14, 23, 24) that TNF-α exerts some of its effects by stimulating production of ROIs in many types of cells. To further confirm that ROIs are involved in IL-6 production in airway epithelial cells, other antioxidants were studied in NHBE and HS-24 cells. DMTU (1 and 10 mM) and 4'-hydroxy-3'-methoxyacetophenone (100 µg/ml) significantly inhibited TNF-α-induced IL-6 production (Fig. 6).

DMSO recovers TNF-α-induced depletion of the intracellular GSH. Treatment with TNF-α significantly decreased the intracellular GSH content of HS-24 cells. Pretreatment of this culture with DMSO recovered the TNF-α-induced decrease in GSH (Table 1).

ROIs are not involved in the production of IL-6 in lung fibroblasts. To determine whether the involvement of ROIs in IL-6 production is specific for bronchial epithelial cells, we tested the ability of X plus XO and H₂O₂ to stimulate IL-6 production in human lung fibroblast cells, WI-38-40. We used lower concentrations of ROIs on WI-38-40 cells because an LDH cytotoxicity assay indicated that WI-38-40 cells were much more sensitive to the cytotoxic effects of ROIs than airway epithelial cells (data not shown). Neither XO nor H₂O₂ increased the production of IL-6 in WI-38-40 cells (Table 2). Next, we stimulated WI-38-40 cells with 200 U/ml of TNF-α in the presence and absence of DMSO and measured the amount of IL-6 in the supernatants at a 24-h time point. TNF-α significantly increased the production of IL-6 in WI-38-40 cells. However, DMSO was not able to inhibit the TNF-α-induced IL-6 enhancement in both protein and mRNA levels (Fig. 7). These results suggest that ROIs may not be involved in the production of IL-6 in human lung fibroblasts.
DISCUSSION

IL-6 is a multifunctional pleiotropic cytokine involved in the modulation of the immune response and inflammation (4). Recent evidence (8, 12, 13, 35) revealed that IL-6 is produced by a variety of cell types in the lung, including alveolar macrophages, lung fibroblasts, endothelial cells, and airway epithelial cells under appropriate stimulation. Increased levels of IL-6 have been detected in the serum and bronchoalveolar lavage fluid in various pathological conditions such as bronchial asthma and idiopathic pulmonary fibrosis (19, 40). In the present study, we investigated the effects of ROIs on the production of IL-6 in human bronchial epithelial cells. XO-derived ROIs, but not H2O2, induced the production of IL-6. Antioxidants significantly suppressed IL-6 release from airway epithelial cells stimulated with TNF-α. These findings

Table 1. Effect of TNF-α and DMSO on GSH concentration in HS-24 cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH Concentration, nmol/10^6 cells</th>
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<tr>
<td>Control</td>
<td>1.00 ± 0.04</td>
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<tr>
<td>DMSO</td>
<td>0.96 ± 0.09</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.81 ± 0.06*</td>
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<tr>
<td>TNF-α + DMSO</td>
<td>1.02 ± 0.01</td>
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Values are means ± SD; n = 3 experiments. HS-24 cells were incubated with tumor necrosis factor-α (TNF-α; 200 U/ml) or TNF-α + DMSO (1%) for 24 h. These cells were then extracted, and total glutathione (GSH) concentrations were determined. *P < 0.01 compared with control and TNF-α + DMSO.

Table 2. Effect of ROI on IL-6 production in lung fibroblasts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IL-6, ng/10^6 cells</th>
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<tr>
<td>Xanthine oxidase</td>
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<tr>
<td>0 µM</td>
<td>0.93 ± 0.01</td>
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<tr>
<td>1.25 µM</td>
<td>0.89 ± 0.01</td>
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<tr>
<td>2.5 µM</td>
<td>0.92 ± 0.01</td>
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<tr>
<td>H2O2</td>
<td></td>
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<tr>
<td>0 µM</td>
<td>1.03 ± 0.01</td>
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<tr>
<td>6.25 µM</td>
<td>0.96 ± 0.01</td>
</tr>
<tr>
<td>12.5 µM</td>
<td>0.98 ± 0.01</td>
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Values are means ± SD; n = 3 experiments. Lung fibroblasts (WI-38-40) cells were incubated for 24 h with xanthine oxidase (+0.7 mM xanthine) or H2O2. Supernatants were harvested and analyzed for interleukin-6 (IL-6) protein by ELISA. ROI, reactive oxygen intermediates.
relevant to ROIs were not observed in human lung fibroblasts.

Our results indicate that XO-derived ROIs induce a dose-dependent increase in IL-6 production in human airway epithelial cells. However, it is difficult to identify the specific ROI responsible for the induction of IL-6 because the various species are highly reactive and often reactive with each other. For instance, O$_2^*$ formed by the action of XO on X is rapidly dismutated to H$_2$O$_2$, which, in turn, is converted in the presence of Fe$^{2+}$ to (OH)$_2$. O$_2^*$ also can contribute to the formation of •OH by converting Fe$^{3+}$ to Fe$^{2+}$. We found that the addition of an O$_2^*$ scavenger, SOD, resulted in the inhibition of the IL-6 response to X plus XO stimulation, suggesting that O$_2^*$ may be, at least in part, involved in IL-6 production in human bronchial epithelial cells. Unexpectedly, H$_2$O$_2$ per se did not seem to be implicated in IL-6 induction. This was also indicated by the result that H$_2$O$_2$ enzymatically generated by the reaction of glucose and glucose oxidase did not enhance IL-6 production (data not shown). Another possibility is that extracellularly generated XO-derived ROIs may penetrate through the cell membranes and change into other kinds of ROIs intracellularly, and it is these intracellularly produced ROIs that induce IL-6 release.

The extent of IL-6 release induced by X plus XO in airway epithelial cells was significant but small compared with that seen in TNF-α-treated cells. It should be considered that ROIs produced by X plus XO may be short-lived and can quickly decompose in medium (9). Presumably, the actual concentrations of ROIs that induced the production of IL-6 in cell cultures were much lower than the theoretical ones.

Consistent with our findings, it has been observed that ROIs are capable of inducing the production of soluble mediators in a variety of cells. Vischer et al. (39) have demonstrated that ROIs induce von Willebrand factor release from human vascular endothelial cells. It has also been reported that ROIs are implicated in high-molecular-weight glycoconjugate secretion from rodent tracheal epithelial cells and in IL-1 decoy receptor release from human myelomonocytic cells (2, 27).

Our results show that antioxidants including DMSO can suppress the induction of IL-6 in response to TNF-α in human bronchial epithelial cells. Generation of ROIs on exposure to a variety of inflammatory stimuli has been reported for many types of cells including bronchial epithelial cells (3, 14, 21, 23, 24). In human endothelial cells, TNF-α and interferon-γ stimulate O$_2^*$ release (23). Lopez et al. (21) have reported that various stimuli, including platelet-activating factor and neutrophil elastase, stimulate bovine bronchial epithelial cells to produce and release H$_2$O$_2$. Our results suggest that intracellular ROIs produced by TNF-α stimulation may be the signals as second messengers to produce IL-6 from bronchial epithelial cells. Our data that TNF-α induced the depletion of intracellular GSH in HS-24 cells and that DMSO recovered this decreased GSH further support this notion. Transcriptional regulatory factors such as NF-κB can be rapidly activated by ROIs (31, 32) and bind to their consensus enhancer sequences in a number of cytokine genes including IL-6 (20). Antioxidants such as DMSO and DMTU have been reported to decrease NF-κB binding activity (31, 34). The findings of our study would support the view that NF-κB activates the IL-6 promoter through oxidants generated by the effects of TNF-α.

The effects of ROIs on IL-6 production appeared to be cell-type specific. Although XO-derived ROIs significantly induced IL-6 production in bronchial epithelial cells, the same oxygen-free radicals had no IL-6 stimulatory effect on WI-38-40 cells. DMSO that is potent in inhibiting IL-6 production in human bronchial epithelial cells did not reduce the production of the same cytokine in WI-38-40 cells. The mechanisms stimulating IL-6 production are diverse (4), and probably lung fibroblasts may use another set of transcription factors to induce IL-6 gene expression than that used in bronchial epithelial cells.

In summary, the present study demonstrates that ROIs are involved in the production of IL-6 in human bronchial epithelial cells. Taken together with the
relationship between ROIs and other cytokines (11), bronchial epithelial cells are actively involved in inflammatory lung diseases on exposure to oxidant burden.

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REFERENCES


