Silica-induced chemokine expression in alveolar type II cells is mediated by TNF-α-induced oxidant stress

EDWARD G. BARRETT, CARL JOHNSTON, GÜNTHER OBERDÖRSTER, AND JACOB N. FINKELSTEIN

Departments of Environmental Medicine and Pediatrics, University of Rochester School of Medicine, Rochester, New York 14642

Barrett, Edward G., Carl Johnston, Günter Oberdörster, and Jacob N. Finkelstein. Silica-induced chemokine expression in alveolar type II cells is mediated by TNF-α-induced oxidant stress. Am. J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L979–L988, 1999.—We have shown previously that epithelial cells may contribute to the inflammatory response in the lung after exposure to crystalline silica through the production of and response to specific chemokines and cytokines. However, the exact cellular and molecular responses of epithelial cells to silica exposure remain unclear. We hypothesize that non-oxidant-mediated silica-cell interactions lead to the upregulation of tumor necrosis factor-α (TNF-α), whereby TNF-α-induced generation of reactive oxygen species (ROS) leads to the activation of the monocyte chemotactic protein (MCP)-1 and macrophage inflammatory protein (MIP)-2 genes. Using a murine alveolar type II cell line, murine lung epithelial (MLE)-15, we measured the early changes in TNF-α, MCP-1, and MIP-2 mRNA species after exposure of the cells to 18 µg/cm² silica (cristobalite) in combination with various antioxidants. Total mRNA was isolated and assayed using an RNase protection assay after 6 h of particle exposure. We found that extracellular GSH could completely attenuate the cristobalite-induced expression of MCP-1 and MIP-2 mRNAs, whereas TNF-α mRNA levels were unaltered. We also found using the oxidant-sensitive dye 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) that treatment of MLE-15 cells with cristobalite and TNF-α (1 ng/ml) resulted in ROS production. This ROS production could be inhibited with extracellular GSH treatment, and in the case of cristobalite-induced ROS, inhibition was also achieved with an anti-TNF-α antibody. The results support the hypothesis that TNF-α mediates cristobalite-induced MCP-1 and MIP-2 expression through the generation of ROS.

INHALATION OF CRYSTALLINE silica particles can result in the development of an inflammatory and fibrotic disease in the lung (10). There is growing evidence that the lung epithelium plays a significant role in silica-induced inflammation by mediating inflammatory cell recruitment through the release of specific chemokines. Previously, using an established cell line and primary rat type II cells in an in vitro silica exposure model, Driscoll and co-workers (5) observed that type II cells respond directly to silica (α-quartz) by increasing expression of macrophage inflammatory protein (MIP)-2 and cytokine-induced neutrophil chemoattractant. More importantly, the same study also showed the localization of MIP-2 mRNA expression in type II cells after in vivo silica exposure (5). We have shown recently that MIP-2, monocyte chemotactic protein (MCP)-1, and RANTES are expressed in a murine alveolar type II cell line after exposure to silica (cristobalite; see Ref. 2). The results of our previous study also indicate that silica-induced expression of type II cell tumor necrosis factor-α (TNF-α) plays a critical role in the upregulation of the MCP-1 and MIP-2 genes (2). The question of how silica-type II cell interactions lead to the expression of TNF-α and how TNF-α subsequently mediates MCP-1 and MIP-2 gene expression remains to be elucidated.

There are two potential mechanisms by which silica-cell interactions may lead to cellular activation; they include 1) cell activation via a distinct activating receptor(s) involved in binding silica particles and/or 2) cell activation by aspects of the ingested particles chemistry, e.g., surface characteristics. In support of the second hypothesis, increasing evidence supports the role for particle-associated reactive oxygen species (ROS) as mediators of pulmonary inflammation and damage after silica exposure (31). ROS can form either on the surface of silica, especially after its fracture (8), or through the generation of a respiratory burst caused by the phagocytosis of silica (30). The addition of the antioxidant N-acetyl-L-cysteine (NAC) has been shown to decrease residual oil fly ash (ROFA) and quartz-mediated interleukin (IL)-8 production by ~50% in normal and TNF-α-primed A549 (epithelial type II cell line) cells (29). Additionally, treatment with the free radical trapper, N-t-buty1-phenyl nitrone, decreases silica-induced expression of TNF-α mRNA in alveolar macrophages and attenuates overall lung injury (9). Both of these studies have speculated that silica-associated components (e.g., transition metals/surface free radicals) mediate oxidant stress and subsequent cellular activation.

We hypothesize, however, that non-oxidant-mediated silica-cell interactions lead to the upregulation of TNF-α, whereby TNF-α-induced generation of ROS leads to the activation of the MCP-1 and MIP-2 genes in type II cells. To test our hypothesis, we exposed a murine lung epithelial (MLE)-15 cell line to cristobalite in combination with various antioxidants (DMSO, extracellular GSH, and NAC) and then measured the changes in TNF-α, MCP-1, and MIP-2 mRNA levels. In addition, we measured the TNF-α-induced expression of TNF-α, MCP-1, and MIP-2 mRNA in MLE-15 cells. We also measured the cristobalite- and TNF-α-induced genera-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
tion of ROS within individual cells using an oxidant-sensitive fluorescent dye and examined whether GSH or anti-TNF-α antibody (cristobalite only) treatment altered this ROS production.

MATERIALS AND METHODS

Particles and reagents. Cristobalite, a form of crystalline silica (gift from Dr. D. Hemenway, University of Vermont, Burlington, VT), was size selected using a cascade cyclone sampler (series 280 Cyclade; Sierra Instruments, Carmel Valley, CA) with an ~1.2-µm particle diameter cutoff. The average particle diameter was measured using a scanning electron microscope equipped with an imaging software package (50 particles were measured and the average was calculated). Because silica particles are not perfect spheres, the average diameter was determined by measuring the longest and shortest diameter of each particle, then averaging the measurements. Mean particle diameter was equal to 0.64 ± 0.05 µm, and particle size ranged from 0.08 to 1.5 µm. Silica was baked at 180°C for 16 h to inactivate any possible endotoxin contamination. Silica particle suspensions were sonicated for 30 s before addition to cell culture exposure experiments. Throughout the studies presented in this paper, we utilized a particle dose of 18 µg/cm² (1.2-µm particle diameter cutoff). The 1.2-µm particle diameter cutoff. The

Cristobalite, a form of crystalline silica (gift from Dr. D. Hemenway, University of Vermont, Burlington, VT), was size selected using a cascade cyclone sampler (series 280 Cyclade; Sierra Instruments, Carmel Valley, CA) with an ~1.2-µm particle diameter cutoff. The average particle diameter was measured using a scanning electron microscope equipped with an imaging software package (50 particles were measured and the average was calculated). Because silica particles are not perfect spheres, the average diameter was determined by measuring the longest and shortest diameter of each particle, then averaging the measurements. Mean particle diameter was equal to 0.64 ± 0.05 µm, and particle size ranged from 0.08 to 1.5 µm. Silica was baked at 180°C for 16 h to inactivate any possible endotoxin contamination. Silica particle suspensions were sonicated for 30 s before addition to cell culture exposure experiments. Throughout the studies presented in this paper, we utilized a particle dose of 18 µg/cm² (1.2-µm particle diameter cutoff). The 1.2-µm particle diameter cutoff. The
glasses containing the MLE-15 cells were removed from their respective culture dishes and then adhered separately to individual glass microscope slides. To maintain cellular viability while viewing under the microscope, several drops of DMEM (no phenol red) were added to the micro cover glasses, and another micro cover glass was placed on top. MLE-15 cells were viewed with fluorescence microscopy and photographed. Images were obtained on an Olympus AX70 Microscope (Olympus America, Lake Success, NY) using Image Pro Plus software (Media Cybernetics, Silver Spring, MD). Samples were epifluorescently illuminated by a 100-watt mercury lamp and were viewed with fluorescence filters (B2E cube). Fields were viewed at ×40 magnification and numerical aperture of 0.85 and were acquired with a charge-coupled device color video camera (DXC-9000; Sony) under computer control with 1/60 s integration time. Illumination caused increased fluorescence because of oxidation of the dye; thus each field was exposed to light for exactly the same time. The average relative fluorescence intensity for 50 cells (cells that were clamped on top of each other or too close together to separate their individual fluorescence were not measured) in three separate experiments was determined as previously described (25) using Image Pro Plus software (Media Cybernetics). Briefly, relative fluorescence intensities for each condition were determined, combined, and partitioned into four brightness classes (1–4). Class 1 represents the lowest fluorescence intensity, and class 4 represents the highest fluorescence intensity.

Statistics. Results from the phosphorimager analysis and ROS quantitation are reported as means ± SE. Statistical comparisons were made using a one-way ANOVA with Tukey-Kramer multiple comparison test, with significance defined as P < 0.05.

RESULTS

Cristobalite and TNF-α induce ROS generation in MLE-15 cells. We used the oxidant-sensitive dye C-DCHDF-DA to detect ROS production in cristobalite- and TNF-α-stimulated MLE-15 cells. After dye uptake, intracellular esterases hydrolyze the ester bonds, releasing the intact nonfluorescent substrate. This reduced substrate is oxidized by ROS to the fluorescent species carboxyfluorescein, which is retained by living cells. MLE-15 cells were stimulated with either cristobalite or TNF-α for 1 or 3 h and then loaded for 20 min with 3 μM C-DCHDF-DA. Cells were examined for fluorescence intensity under a fluorescence microscope. Figure 1 shows the fluorescence images of MLE-15 cells after C-DCHDF-DA staining. Control cells exhibited a low intensity of fluorescence (Fig. 1, A, B, A1, and B1). In contrast, both cristobalite- and TNF-α-stimulated cells showed an increased level of fluorescence (Fig. 1, C–F and C1–F1). TNF-α-induced fluorescence appeared to peak after 1 h and then decrease, whereas cristobalite did not induce fluorescence until 3 h of exposure. Preincubation of the cells for 0.5 h with 50 mM extracellular GSH reduced the cristobalite- and TNF-α-induced fluorescence to near control levels (Fig. 1, G, H, G1 and H1). Also, treatment with an anti-TNF-α antibody reduced the cristobalite-induced fluorescence to control levels (Fig. 1, I and I1). Treatment with extracellular GSH or anti-TNF-α alone led to fluorescence levels similar to those of untreated controls (data not shown).

Cristobalite-induced ROS generation mediates chemokine mRNA expression. Using various antioxidants and modifiers of cellular oxidant status in the presence or absence of cristobalite, we examined whether cytokine and chemokine expression in MLE-15 cells could be altered. Treatment with the hydroxyl scavenger DMSO, extracellular GSH, or NAC decreased cristobalite-induced MIP-2 and MCP-1 mRNA levels by 81 and 49%, 99 and 97%, and 70 and 53%, respectively (Fig. 2, A–C). Treatment with BSO, which inhibits γ-glutamylcysteine synthetase and subsequently reduces intracellular GSH levels, also led to a reduction in cristobalite-induced MIP-2 (42%) and MCP-1 (42%) mRNA levels.

GSH-mediated regulation of cristobalite, TNF-α, and IFN-γ-induced cytokine and chemokine mRNA expression. As shown in Figs. 1 and 2, extracellular GSH can inhibit cristobalite- and TNF-α-induced ROS production and attenuate cristobalite-induced MCP-1 and MIP-2 mRNA production in MLE-15 cells. To further support our hypothesis that cristobalite-induced chemokine expression is mediated by TNF-α-induced ROS, we examined MCP-1, MIP-2, and TNF-α mRNA levels in MLE-15 cells after treatment with cristobalite or TNF-α in combination with extracellular GSH. We found that both cristobalite and TNF-α could elevate TNF-α mRNA levels; however, on coincubation with extracellular GSH, only TNF-α-induced TNF-α mRNA was completely inhibited (Fig. 3, A and B). In contrast, MCP-1 and MIP-2 mRNA levels were completely attenuated after treatment with cristobalite or TNF-α in combination with extracellular GSH (Fig. 4, A–C). Interestingly, even when BSO was used to deplete intracellular GSH levels, the addition of extracellular GSH greatly reduced cristobalite-induced TNF-α mRNA levels (Fig. 3, A and B). Also, cristobalite-induced MCP-1 and MIP-2 mRNA levels were completely attenuated after BSO and extracellular GSH treatment (Fig. 4, A–C).

We have shown previously (3) that IFN-γ can synergistically elevate cristobalite-induced MCP-1 mRNA levels but has no effect on TNF-α or MIP-2 mRNA levels in MLE-15 cells. In the present study, we found that treatment with extracellular GSH had only a slight effect on cristobalite-IFN-γ-induced MCP-1 mRNA levels (Fig. 4, A–C). Interestingly, the addition of IFN-γ in combination with cristobalite and extracellular GSH appears to inhibit the expected expression of TNF-α mRNA in MLE-15 cells (Fig. 3, A and B).

DISCUSSION

We have shown previously, using an anti-TNF-α antibody, that cristobalite-induced activation of the MCP-1 and MIP-2 genes in the MLE-15 cells is mediated by TNF-α (2). This finding is further supported by the results of another study that found that passive immunization of mice against TNF-α markedly attenuates the increases in lung MIP-2 mRNA seen in response to α-quartz (4). Thus, in the present study, we set out to better characterize the mechanisms through which cristobalite-type II cell interactions lead to increased chemokine expression. We hypothesized that...
Fig. 1. Detection of reactive oxygen species production after cristobalite (SC) or tumor necrosis factor-α (TNF-α) exposure in murine lung epithelial-15 cells loaded with 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate di(acetoxymethyl ester). A: untreated 1-h control; B: untreated 3-h control; C: SC 1-h exposure; D: SC 3-h exposure; E: TNF-α 1-h exposure; F: TNF-α 3-h exposure; G: SC + extracellular GSH 3-h exposure; H: TNF-α + extracellular GSH 1-h exposure; I: SC + anti-TNF-α antibody (10 µl/ml); A1–I1: relative fluorescence intensities for each corresponding condition determined as described in MATERIALS AND METHODS. For all exposures, SC = 18 µg/cm², TNF-α = 1 ng/ml, and extracellular GSH = 50 mM (0.5-h pretreatment before addition of SC or TNF-α). Images are representative of 3 separate experiments. Con, control.
Fig. 1.—Continued.
non-oxidant-mediated cristobalite-cell interactions lead to the upregulation of TNF-α, whereby TNF-α-induced generation of ROS leads to the activation of the MCP-1 and MIP-2 genes in type II cells. In support of this hypothesis, we have found that the cristobalite-induced

Fig. 3. A: measurement of TNF-α mRNA after various treatments (6-h exposure) of SC, TNF-α, and interferon-γ (IFN-γ) in combination with extracellular GSH using the RNase protection assay; SC = 18 µg/cm², TNF-α = 1 ng/ml, IFN-γ = 100 U/ml, extracellular GSH = 50 mM, and BSO = 0.4 mM. B: quantitative assessment of TNF-α mRNA levels from RNase protection analysis was carried out with a phosphorimager. Shown are means ± SE of 3 separate experiments. All values were normalized using constitutively expressed GAPDH mRNA levels. *P < 0.05, treatment vs. SC; **P < 0.05, treatment vs. TNF-α; †P < 0.05, treatment vs. TNF-α + SC; ††P < 0.05, treatment vs. IFN-γ + SC.

Fig. 2. Measurement and quantitation of SC-induced chemokine mRNA expression after treatment with various antioxidants and tyrosine kinase inhibitors. A: results of RNase protection assay where SC = 18 µg/cm², extracellular GSH = 50 mM, DMSO = 1%, N-acetyl-L-cysteine (NAC) = 30 mM, and dl-buthionine-[S,R]-sulfoximine (BSO) = 0.4 mM. Quantitative assessment of macrophage inflammatory protein (MIP)-2 (B) and macrophage chemotactic protein (MCP)-1 (C) mRNA levels from RNase protection analysis was carried out with a phosphorimager. Shown are means ± SE of 3 separate experiments. All values were normalized using constitutively expressed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels. *P < 0.05, treatment vs. SC.
generation of ROS in MLE-15 cells can be attenuated by treating the cells with an anti-TNF-\(\alpha\) antibody. In addition, pretreatment with extracellular GSH inhibits cristobalite- and TNF-\(\alpha\)-induced production of ROS. At the level of gene expression, we found that treatment with various antioxidants inhibits cristobalite- and TNF-\(\alpha\)-induced expression of MCP-1 and MIP-2 mRNAs in MLE-15 cells. More importantly, we show that antioxidant treatment does not inhibit the direct cristobalite-induced expression of TNF-\(\alpha\) mRNA. Although the level of cristobalite-induced TNF-\(\alpha\) mRNA appears to be lower after antioxidant treatment, we believe this is due to the inhibition of secondarily produced TNF-\(\alpha\) autocrine interactions and not the inhibition of the initial cristobalite-cell interactions. We have also shown that TNF-\(\alpha\)-induced TNF-\(\alpha\) mRNA can be completely inhibited with extracellular GSH pretreatment.

Our results compliment earlier findings by Stringer and Kobzik (29) that show that antioxidant treatment can attenuate the \(\alpha\)-quartz-induced production of IL-8 in A549 cells. However, our proposed mechanism of silica-induced oxidant stress and cellular activation differs. The same investigators have shown that silica and other environmental particulates (e.g., ROFA, TiO\(_2\), and iron oxide) can interact with type II cells and alveolar macrophages via scavenger-type receptors (22, 28). Thus the investigators hypothesize that, because the scavenger receptors mediate uptake of both inert (e.g., TiO\(_2\)) and proinflammatory (\(\alpha\)-quartz) particles without evidence of receptor-mediated cell activation, then particle-associated components (e.g., transition metals or surface free radicals) are likely to mediate intracellular oxidant stress and proinflammatory activation (28). Interestingly, the same study shows that inhibitors of the scavenger receptors, polyinosinic acid and heparin, only inhibited \(\alpha\)-quartz binding to A549 cells by \(\sim\) 35 and \(\sim\) 45%, respectively (28). The authors concede in their discussion that multiple receptor types are likely to contribute to particle binding and uptake by epithelial cells. Our results in conjunction with these previous studies (22, 28) support the idea that non-oxidant-mediated cristobalite-cell interactions (e.g., receptor binding) may lead to the upregulation of TNF-\(\alpha\), whereby TNF-\(\alpha\)-induced generation of ROS leads to the activation of MCP-1 and MIP-2 genes in type II cells. It should be noted that other non-receptor-mediated particle-cell interactions could also potentially mediate the observed cellular activation. However, to our knowledge, there is no evidence in the literature that describes the simple physical act of

Fig. 4. A: measurement of MIP-2 and MCP-1 mRNAs after various treatments (6-h exposure) of SC, TNF-\(\alpha\), and IFN-\(\gamma\) in combination with extracellular GSH using the RNase protection assay; SC = 18 \(\mu\)g/cm\(^2\), TNF-\(\alpha\) = 1 ng/ml, IFN-\(\gamma\) = 100 U/ml, extracellular GSH = 50 mM, and BSO = 0.4 mM. Quantitative assessment of MIP-2 (B) and MCP-1 (C) mRNA levels from RNase protection analysis was carried out with a phosphorimager. Shown are means \(\pm\) SE of 3 separate experiments. All values were normalized using constitutively expressed GAPDH mRNA levels. *\(P < 0.05\), treatment vs. SC; **\(P < 0.05\), treatment vs. TNF-\(\alpha\); †\(P < 0.05\), treatment vs. TNF-\(\alpha\) + SC; ††\(P < 0.05\), treatment vs. IFN-\(\gamma\) + SC; \(\phi\)\(P < 0.05\), treatment vs. IFN-\(\gamma\).
non-receptor-mediated endocytosis causing cellular activation. Future studies need to be conducted to identify what other receptor- or non-receptor-mediated particle-cell events lead to cellular activation.

In contrast, if the alternative hypotheses that silica-associated free radicals and/or a silica-induced respiratory burst are responsible for initiating the expression of MCP-1, MIP-2, and TNF-α, we should have observed the following results instead: 1) treatment with an anti-TNF-α antibody does not alter cristobalite-induced ROS or MCP-1 and MIP-2 mRNA levels and 2) antioxidant treatment not only inhibits cristobalite-induced MCP-1 and MIP-2 mRNA expression but also inhibits TNF-α mRNA. However, we are not suggesting that this alternative hypothesis is never involved in silica-induced activation of type II cells. The fact that we used “aged” cristobalite, which contains very few surface free radicals in comparison with freshly fractured silica (8), could explain why we did not observe a direct silica- and ROS-mediated activation of the MLE-15 cells.

The idea that TNF-α leads to oxidant production and subsequent gene activation is not new. Evidence exists showing that antioxidants inhibit TNF-α-mediated stimulation of IL-8, MCP-1, and collagenase expression in cultured human synovial cells (26). Also, a previous study utilizing human endothelial cells indicated that TNF-α induces nuclear factor-κB activation and the resultant E-selectin gene expression by a pathway that involves formation of ROS and that E-selectin expression can be inhibited by the antioxidant NAC (25). In addition, TNF-α-induced expression of cyclooxygenase-2 in rat mesangial cells can be inhibited with antioxidants and inhibitors of NADPH oxidase (6). However, the exact mechanism whereby TNF-α triggers oxidant production in type II cells is not known.

The effects of ROS within the lung are counterbalanced by a complex system of enzymatic and nonenzymatic antioxidants located both intracellularly and extracellularly (14). Little is known about the antioxidant status of the lung after exposure to silica and the involvement of individual antioxidants in lung defense against silica-induced cell injury. Previous studies in rats have shown that, after inhalation of silica, mRNA levels for the antioxidant enzymes catalase and manganese-containing superoxide dismutase (Mn SOD) are elevated in lung homogenates (19, 20). More specifically, another study found that silica-induced increases in Mn SOD gene expression are localized to type II epithelial cells and alveolar macrophages (16).

GSH plays a major role in the antioxidant system by acting as a substrate for GSH peroxidase. GSH peroxidase utilizes GSH as a reductant to reduce toxic peroxides (21). Depletion of GSH potentiates cellular injury due to oxidant stress (24), and stimulation of processes that support maintenance of GSH protects against injury (1, 3). In the present study, we utilized extracellular GSH and NAC to examine the role of ROS in cristobalite-induced cytokine and chemokine expression. Previous investigators have shown that extracellular GSH can elevate intracellular GSH levels and protect alveolar macrophages (7), intestinal epithelial cells (23), and kidney cells (12) from oxidative injury. NAC is a thiol compound that can act as a cysteine source for the repletion of intracellular GSH and act as a direct scavenger of ROS (1, 3). Our results showed that extracellular GSH and NAC (cristobalite gene activation) can inhibit cristobalite- and TNF-α-induced oxidant stress and gene activation (e.g., MIP-2 and MCP-1) in MLE-15 cells. These results suggest that GSH levels may play an important role in mediating the cristobalite- or TNF-α-induced response. Future experiments should be performed to examine the exact role GSH levels play in mediating the cristobalite- and TNF-α-induced response in type II cells.

One might speculate that a decrease in intracellular GSH would lead to an increase in cristobalite-induced ROS and subsequently an increase in MCP-1 and MIP-2 mRNA levels. Interestingly, we found that, even though BSO treatment in combination with cristobalite led to a significant decrease in total GSH levels (unpublished results), cristobalite-induced MCP-1 and MIP-2 mRNA levels actually decreased. A simple explanation may be that after BSO treatment the cells become unable to respond efficiently to oxidant stress, and the addition of cristobalite, which is already cytotoxic to the MLE-15 cells (2), just leads to an increase in cell death (24). Thus fewer cells are able to respond to cristobalite treatment by elevating cytokine and chemokine levels. Treatment with t-butylhydroquinone (TBHQ), a monofunctional phase II enzyme inducer that produces ROS in combination with BSO, has been shown to elevate LDH levels above TBHQ treatment alone in the rat lung epithelial L2 cell line (24).

We have also found that the addition of extracellular GSH to BSO- and cristobalite-treated MLE-15 cells can still completely attenuate the cristobalite-induced expression of MCP-1 and MIP-2. Although several authors, mostly from the same research group, have described uptake of intact GSH by type II cells (13), we found that MLE-15 cells cannot (unpublished results). Others have also found it difficult to demonstrate any uptake of intact GSH in type II cells (32). The depletion of GSH in type II cells has been shown to elevate γ-glutamyltransferase activity (33), which would subsequently elevate intracellular cysteine levels, via the breakdown of extracellular GSH, in an effort to replete intracellular GSH levels. Even though the synthesis of new GSH is blocked in our experiment, an elevation in cysteine, which has antioxidant activity itself (17, 27), could explain why the cristobalite-induced chemokine response is still attenuated in the presence of extracellular GSH and BSO.

Previously, we have shown that IFN-γ synergistically elevates cristobalite-induced MCP-1 mRNA levels without influencing MIP-2 mRNA levels (2). In the present study, we have found that extracellular GSH in combination with IFN-γ-cristobalite treatment inhibited MIP-2 mRNA expression, whereas MCP-1 mRNA levels were only minimally altered. This lends further sup-
port to our hypothesis that IFN-γ can enhance the cristobalite-induced expression of MCP-1 through a pathway independent of TNF-α and the generation of ROS. Interestingly, IFN-γ either alone or in combination with cristobalite has no effect on TNF-α mRNA expression. However, IFN-γ-cristobalite treatment in the presence of extracellular GSH leads to the inhibition of TNF-α mRNA levels. The cellular and molecular mechanisms contributing to this observation are unknown.

In summary, the present study shows that non-oxidant-mediated cristobalite-cell interactions lead to the upregulation of TNF-α, whereby TNF-α-induced generation of ROS leads to the activation of the MCP-1 and MIP-2 genes. In addition, in the presence of IFN-γ, other non-TNF-α/oxidant-mediated pathways appear to be activated after cristobalite exposure. These results support the idea that silica-cell interactions can lead to cellular activation through multiple pathways (oxidant/nonoxidant mediated).

This work was supported in part by National Institute of Environmental Health Sciences Grants ES-04872, ES-01247, and ES-07026 and Center for Indoor Air Research Grants CA-27791 and CA-11051. Address for reprint requests and other correspondence: J. N. Finkelstein, Box 777, Dept. of Pediatrics, Univ. of Rochester School of Medicine, Rochester, NY 14642 (E-mail: finj@ehsct7.envmed.rochester.edu).

Received 2 December 1998; accepted in final form 22 February 1999.

REFERENCES


