Induction of Chemokines by Low-Dose Intratracheal Silica Is Reduced in TNFR I (p55) Null Mice

Gloria S. Pryhuber,*†‡ Heidie L. Huyck,* Raymond Baggs,‡ Günter Oberdörster,‡ and Jacob N. Finkelstein*†

*Department of Pediatrics and ‡Department of Environmental Medicine, Strong Children’s Research Center, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

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Previous studies suggest that tumor necrosis factor alpha (TNF-α) and the TNFR I (p55) and TNFR II (p75) receptors mediate the pulmonary fibrotic response to silica. In order to further define the role of the TNFRI (p55) receptor in induction of profibrotic chemokines by low-dose silica/crystalline silica (50 μg/50 μl/mouse) or control diluent saline was instilled into the trachea of TNFRI gene ablated (−/−) and C57BL/6 (WT) control mice. Lung tissue was harvested and bronchoalveolar lavage (BAL) performed 24 h and 28 days following silica administration. Selected profibrotic chemokine mRNAs were quantified by ribonuclease protection assay, normalized to ribosomal protein L32 mRNA content and expressed relative to saline control treated lungs. Induction of MIP-1β, MIP-1α, MIP-2, IP-10, and MCP-1 mRNAs was attenuated in the TNFRI−/− mice, in comparison to WT mice, particularly at 28 days after exposure. ELISA assays for MIP-1α and MIP-2 in homogenized lung tissue similarly demonstrated marked induction of both chemokines 24 h after silica treatment, which was persistent at 28 days in WT but not in TNFRI−/− mice. The percentage of BAL cells that was neutrophils was comparably increased in WT and RI−/− lungs at 24 h (49 ± 12% vs. 46 ± 10%) and 28 days (6.2 ± 1.5% vs. 4.5 ± 1%). The increase in total lavagable cells and BAL protein was also independent of strain. Histology revealed mild alveolitis without granuloma formation, and fibrosis was sustained at 28 days after treatment in WT but not in TNFRI−/− mice. Silica dependent recruitment of neutrophils to the alveolar space and alveolar protein leak were, however, not altered by the absence of the TNF receptor.

Key Words: silica; tumor necrosis factor α (TNF-α); TNF receptor; chemokine; intratracheal.

Inhalation of crystalline silica induces pulmonary inflammation and fibrosis in humans with occupational exposure. Exposure to high doses of silica causes a chronic inflammatory lung disease, silicosis, but silica is also implicated in more subtle illnesses, including small airway disease, bronchitis, emphysema, immune-mediated diseases, vascular diseases, and glomerulonephritis (Ding et al., 2002). The mechanisms responsible for long-term immunologic responses to crystalline silica are poorly understood.

Acute silicosis is characterized by activation of resident macrophages that are seen to engulf the silica particles, as well as by a rapid and persistent influx of polymorphonuclear cells. Leukocytes are recruited and activated by direct chemoattractant properties of the silica particles, as well as by particle-mediated induction of complement, pro-inflammatory lipids, such as leukotriene B4, and chemokines. Chemokines, a superfamily of small proteins (6–8 kD), released in response to inflammatory stimuli, propagate the inflammatory response. MIP-1α, MCP1, and RANTES are C-C chemokines, with no amino acid intervening between the first two conserved cysteines, which primarily attract monocytes and macrophages, induce IL-1, IL-6, and TNF-α and that modulate T-lymphocyte recruitment and type switching (Olszewski et al., 2000). Human MIP-1α is primarily a monocyte chemoattractant, while murine MIP-1α attracts PMNs as well as monocytes. MIP-1α has been associated with chronic inflammatory disease including rheumatoid arthritis and interstitial lung disease. MIP-2, a murine homologue of the human GRO genes and classified in the C-X-C family with one amino acid between the first two conserved cysteines, is a potent chemoattractant and activator of neutrophils (PMNs). Both MIP-1α and MIP-2 have been implicated in the pathogenesis of silica-induced pulmonary inflammation. They are each induced in rat lung following intratracheal instillation of silica prior to the accumulation of pulmonary inflammatory cells (Driscol, 1993). In particular, increased expression of MIP-2 mRNA was detected in epithelial cells of the terminal bronchioles and alveolar ducts, as well as in cells consistent with alveolar epithelial cells and alveolar macrophages. Pretreatment with anti-MIP-2 antisera markedly reduced the silica-induced neutrophil recruitment measured in BAL, suggesting an important role of the chemokine in silica-induced lung inflammation (Driscol et al., 1996).

In animal models, the severity of the pulmonary inflammatory response to instilled crystalline silica correlates with the induction of tumor necrosis factor alpha (TNF-α) gene expres-
sion. Following exposure to silica, the measurable increase in TNF-α mRNA and protein in lung and bronchioalveolar lavage of different strains of mice correlates positively with the sensitivity of the strain to silicosis. Treatment with anti-TNF-α antibodies or soluble receptors ameliorated subsequent silica-induced disease (Ohutsuka et al., 1995; Piguet et al., 1990; Piguet and Vesin, 1994). Many of the changes measured in lung after silica exposure can be accounted for by increased TNF-α activity, including recruitment of polymorphonuclear cells and activation of alveolar macrophages. TNF-α may also be responsible for the induction of chemokines in the lung in response to silica. Studies by Barrett et al. demonstrated that, in a pulmonary epithelial cell line, silica-induced increase in chemokine expression was blocked by the presence of anti-TNF-α antibodies, suggesting that the chemokine response is not due to the direct oxidizing effect of silica particles, but that it is mediated by TNF-α (Barrett et al., 1999b).

Consistent with the studies with anti-TNF-α antibodies, both in vitro and in vivo, the transgenic ablation of both TNF-α receptors, TNFR1 (p55) and TNFRII (p75), resulted in reduced fibrogenic effect of endotracheal silica (Ortiz et al., 1999). The role of each receptor in induction of chemokines and neutrophilic infiltration has not been determined. In the present study, TNF receptor p55 knockout in a C57BL/6 background, and C57BL/6 control mice were exposed to approximately 2 mg/kg crystalline silica, a relatively low dose in comparison to the majority of experimental silica models, in order to determine the role of this TNF receptor in the induction and maintenance of a silica-induced, pulmonary inflammatory response.

MATERIALS AND METHODS

Animals. The TNFRI−/− strain was generated by homologous recombinant in C57BL/6-derived embryonic stem cells (ImmuGene, Seattle, WA) as previously described (Peschon et al., 1998). Age and sex matched, 8–10-week-old control, C57BL/6, and TNFRI−/− mice were purchased from the Jackson Laboratories (Bar Harbor, ME) and maintained in micro-isolator cages in specific pathogen-free rooms at the University of Rochester Medical Center. All animal care and experimental protocols follow the guidelines of the IACUC.

Intratracheal silica exposure. Crystalline silica (cristobalite, a gift from Dr. D. Hemenway, University of Vermont, Burlington, VT) was size-selected by cascade cyclone sampler (Series 280 Cyclade, Sierra, Instruments Inc., Carmel Valley, CA) with an approximate 1.2 μm-particle diameter cutoff. The mean particle size, and size range, of the resulting silica preparation was 0.64 ± 0.05 μm and 0.08–1.5 μm, respectively, as measured by scanning electron microscopic analysis of 50 particles. The silica particles were baked at 180°C for 16 h to inactivate any contaminating endotoxin prior to suspension by sonication in nonpyrogenic saline. Silica (50 μg/50 μl per mouse, approximately 2 mg/kg, n = 4/group) or control saline was delivered by blunt-needle endotracheal intubation under light isoflurane anesthesia. Lung tissue was isolated at 24 h and 28 days after silica treatment.

Histology. The left lung was inflation-fixed with 2% glutaraldehyde in 0.1 M cacodylic acid at 10-cm H₂O pressure for 24 h, dehydrated, and paraffin-embedded. Sections (4 μm) were analyzed by Gomori’s trichrome stain and light microscopy. A toxicity score of 0 (no histopathology) to 4+ (severe abnormalities) was assigned to each tissue based on the degree of alveolitis, bronchiolitis, bronchitis, fibrosis, and extent of involvement, as previously described (Adawi et al., 1998).

Ribonuclease protection assay. Total cell RNA was extracted by Phase Lock Gel II columns (5Prime-3Prime, Boulder, CO) from lung homogenized and lysed in 4 M guanidinium isothiocyanate (Kodak Chemical Co., Rochester, NY), 0.5% N-lauryl sarcosine, and 25 mM sodium citrate (Sigma) as previously described (Pryhuber et al., 2000). The ribonuclease protection assay reactions (RPA) were performed with commercial reagents and protocols (RiboquantTM, Pharmingen, Inc., San Diego, CA). Radiolabeled, single-strand RNA probes from template mCK5 were synthesized at room temperature, utilizing (α–32P)-UTP (3000 Ci/mmol EasyTideTM, New England Nuclear) and T7 polymerase. RNA samples (5 μg by absorbance at 260 nm), including murine RNA and yeast 18S (2 μg) as positive and negative controls, respectively, dried, then resuspended in 8 μl of hybridization buffer plus 2 μl radiolabeled probe (3 × 10⁶ cpm/μl). The samples were denatured at 90°C and incubated overnight at 56°C. Following incubation, single-strand RNA was digested in an RNase A/T1 cocktail, followed by proteinase K digestion. The protected, radiolabeled RNA fragments were resolved on a 6% acrylamide/urea gel (Gibco), utilizing radiolabeled probe (1000–2000 cpm) as size markers. The intensity of protected probe bands in dried gels was quantified by phosphorimaging (Molecular Dynamics, Sunnyvale, CA) and normalized to rpL32 mRNA content of each sample.

Bronchoalveolar lavage. The trachea was intubated and the lungs isolated prior to exhaustive lavage with warmed normal saline (1 ml × 10). Total protein was determined in the first two milliliters of BAL by bichinchoninic acid (BCA) assay, utilizing commercial reagents and protocol (Pierce, Inc., Rockford, IL) (Smith et al., 1985). LDH (nmol/min/ml) was assayed utilizing a commercial diagnostics kit (Sigma, St. Louis, MO). All 10 lavage results were pooled and centrifuged at 250 × g for 10 min at 4°C. The resulting BAL cell pellet was diluted. Aliquots were analyzed by hemocytometer and trypan blue dye exclusion for viable cell count and by light microscopy of Diff-Quik-stained (Dade Behring AG, Inc., 400 cells counted) cytospin preparations for cell differential.

MIP-1α and MIP-2 ELISAs. Total cell protein was isolated from the left lung by homogenization in 50 mM Tris (pH 7.4), 150 mM sodium chloride, 2 mM EDTA, 25 mM sodium fluoride, 25 mM β-glycerol phosphate, 0.1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, 0.2% Triton X-100, 0.3% Igepal CA-630, 10 μg/ml leupeptin, 10 μg/ml pepstatin, and 10 μg/ml aprotonin. Following the determination of total protein by BCA assay, the samples were stored at ~8°C. MIP-1α and MIP-2 ELISAs were performed using 50 μl total cell protein following provided protocol (Quantikine, R&D Systems, Minneapolis, MN). These assays are designed for cell culture homogenates or serum samples. Components of the more complex lung homogenate therefore may interfere with the measure of the chemokines by ELISA. However, there is no evidence that the matrix of the WT versus TNFRI−/− lung homogenates differ significantly at these low silica doses and time points so that the ELISA results obtained can be compared between strains.

Statistical analysis. Quantitative RPA and ELISA data were analyzed by single factor ANOVA and Fischer’s protected least significant difference (PLSD) statistic, utilizing Statview 4.0 statistical analysis software (SAS Institute, Cary, NC).

RESULTS

Both WT and TNFRI−/− animals tolerated intratracheal administration of either saline or silica without clinical signs of morbidity or mortality.

Induction of chemokine gene expression following intratra- cheal silica. Changes in chemokine gene expression in adult WT and TNFRI−/− mice exposed to intratracheal diluent saline or to silica (50 μg/mouse, approximately 2 mg/kg) were de-
determined by ribonuclease protection assay of homogenized lung tissue harvested either for 24 h or 28 days after silica instillation. A representative RPA and the quantitative analysis of cumulative data normalized to total RNA concentration are displayed in Figure 1. RANTES was constitutively expressed in lungs of both strains and was not significantly altered by silica exposure. Messenger RNA for MIP-1β, MIP-1α, MIP-2, IP-10, and MCP-1 were increased 2- to 4-fold in WT animals 24 h after silica treatment and remained significantly elevated 28 days after treatment. The fold increase in chemokine gene expression was significantly greater in WT as compared to TNFRI−/− at both 24 h and 28 days (Fig. 1B). MIP-1β, MIP-1α,
MIP-1α, IP-10, and MCP-1 mRNAs were increased in TNFRI−/− animals 24 h after silica dosing, but unlike the WT animals chemokine induction was not sustained at 28 days. In order to demonstrate the change in chemokine levels in WT and TNFRI−/− animals relative to expression in the lungs prior to silica exposure, the MIP-1α and MIP-2 mRNA levels were analyzed relative to ribosomal protein rpl32, a housekeeping gene that was not altered in response to silica. As demonstrated in Figures 2A and 3A, MIP-1α and MIP-2 to rpl32 mRNA ratios were markedly elevated in WT animals 24 h after silica exposure with no resolution toward basal levels, even 28 days after treatment. In mice lacking TNFRI gene expression, MIP-1α and MIP-2 were also significantly induced within 24 h of treatment. There was no significant difference between MIP-1α mRNA levels in WT versus TNFRI−/− lungs 24 h after silica instillation. In contrast, induction of MIP-2 mRNA was greater in WT than in TNFRI−/− at 24 h after silica treatment. By 28 days after silica delivery, both chemokine mRNAs returned to near basal levels in the TNFRI−/− mice, levels that were significantly lower than those measured in WT animals at this time point.

**Induction of MIP-1α and MIP-2 protein in whole lung following intratracheal silica.** In order to determine if the induction of chemokine mRNA by silica reflects an increase in chemokine protein, ELISA assays for MIP-1α and for MIP-2 were performed on homogenized whole lung samples prepared from WT and TNFRI−/− lungs 24 h and 28 days after silica treatment. As demonstrated in Figures 2B and 3B, lung MIP-1α and MIP-2 protein contents, expressed per μg of total lung homogenate protein, were markedly elevated in WT animals 24 h after treatment. MIP-1α increased approximately 2-fold, while MIP-2 protein was increased approximately 10-fold. At 28 days, MIP-1α and MIP-2 remained approximately 4- and 10-fold, respectively, greater in silica treated WT lungs when compared to saline controls.

In mice lacking TNFRI gene expression, MIP-1α and MIP-2 were induced within 24 h of treatment; approximately 3-fold each. As demonstrated at the mRNA level, there was no significant difference between MIP-1α protein levels in WT versus TNFRI−/− mice at 24 h after silica. However, induction of MIP-2 protein was statistically greater in WT than in TNFRI−/− at 24 h after silica instillation. Also similar to relative changes in mRNA, both chemokine proteins returned to near basal levels in the TNFRI−/− mice by 28 days after silica delivery, in contrast to WT animals in whom MIP-1α and MIP-2 proteins remained significantly elevated at 28 days.

**Histological evidence of inflammation induced by intratracheal silica.** A chronic, granulomatous inflammation is well described in rodents treated intratracheally with greater than 50 mg/kg crystalline silica. In the present study, sections were analyzed following H&E staining as well as Gomori’s trichrome stain, (data not shown) for degree of inflammation and fibrosis (Fig. 4). Lung injury and fibrosis score was zero in saline-treated control animals. Following silica treatment, inflammation was clearly evident, but only mild to moderate in severity and without granuloma formation, which is consistent with the dose utilized being approximately 10-fold lower than previously reported. Septal thickening and a hypercellularity, consistent with the presence of neutrophils and mononuclear inflammatory cells, were evident at both 24 h and at 28 days after silica. Assignment of lung injury and fibrosis scores by an independent, blinded observer (R.B.) suggested a trend toward less severe response in the TNFRI−/− lungs; however, morphologic evidence suggested overall mild injury (Fig. 4C).
Bronchoalveolar lavage cell count and total protein. In order to further characterize the inflammatory response to low-dose silica in WT versus TNFRI⁻/⁻ mice, the percentage of lavagable cells that were macrophages decreased to approximately 50% at 24 h, and remained mildly reduced at 90%, 28 days after silica instillation (Fig. 5B). The change in percent macrophages was inversely related to a 50% increase in the percentage of neutrophils at 24 h and the notable persistence of 5% neutrophils at 28 days (Fig. 5C). The increase in neutrophils was independent of the presence of TNFRI. A modest increase in lavagable lymphocytes was noted at 28 days after silica treatment that was also independent of TNFRI function (Fig. 5D).

BAL total protein levels were significantly increased in all animals of both WT and TNFRI⁻/⁻ groups 24 h after silica treatment, and remained elevated 28 days after treatment (Fig. 6A). There was, however, no significant difference between WT and TNFRI⁻/⁻ BAL total protein levels in response to the tested dose of silica. BAL LDH was increased in WT animals 28 days after silica treatment \((p < 0.01)\). A similar trend was noted 24 h after treatment but was not statistically significant (Fig. 6B). In TNFRI⁻/⁻ animals, BAL LDH was not significantly elevated at either time point.

Based on the morphology of cells obtained by BAL prepared for analysis by Cytospin and Diff-Quick stain in both WT and TNFRI⁺/⁺ mice, the percentage of lavagable cells that were macrophages decreased to approximately 50% at 24 h, and remained mildly reduced at 90%, 28 days after silica instillation (Fig. 5B). The change in percent macrophages was inversely related to a 50% increase in the percentage of neutrophils at 24 h and the notable persistence of 5% neutrophils at 28 days (Fig. 5C). The increase in neutrophils was independent of the presence of TNFRI. A modest increase in lavagable lymphocytes was noted at 28 days after silica treatment that was also independent of TNFRI function (Fig. 5D).

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DISCUSSION

Crystalline silica is a prevalent particulate in certain workplaces, generated by crushing and processing of certain rocks and sand. Exposure to silica has been associated with numerous pulmonary and immunologic diseases, yet the mechanisms of injury and activation of the inflammatory reaction are not well understood (Cooper et al., 2002; Ding et al., 2002). Rodent models of silica instillation develop a granulomatous, fibrotic pathology within one month of exposure to silica, with characteristics of the fibroproliferative disease seen in human clinical silicosis. These experimental models of silica-induced lung injury utilize relatively large doses of endotracheal crystalline silica (i.e., 50 to 200 mg/kg in mice) or prolonged exposures to lower doses by inhalation (Driscoll et al., 1990, 1991). The threshold and duration of inflammation due to exposure to low dose silica is less well defined. The present study demonstrates an increase in intra-alveolar protein leak, polymorphonuclear cell recruitment, and whole-lung chemokine induction in C57BL/6 mice following a relatively low dose (50 μg/mouse) of crystalline silica. This raises the possibility of low-grade pulmonary inflammation in exposed humans. The significance of this prolonged inflammatory response, relative to susceptibility to secondary lung disease or systemic illness, is unclear.

Induction of inflammation and fibrosis in response to silica is closely tied to the activity of TNF-α and its receptors, TNFR1 and TNFR2. Exposure of macrophages to silica induced TNF-α mRNA and protein (Barrett et al., 1999a; Claudio et al., 1995). In vivo studies utilizing TNF receptor gene ablation models and anti-TNF antibodies or soluble receptors demonstrated attenuation of silica-induced fibrogranulomatous disease with inhibition of TNF-α activity (Piguet et al., 1990; Piguet and Vesin, 1994). Genetic studies have associated occurrence and severity of silicosis in miners with specific polymorphisms of the TNF-α gene (Yucesoy et al., 2002). The current study demonstrates in vivo that silica-dependent induction of chemokines, including MCP-1, IP-10, MIP-1β, MIP-2 and MIP-1α, is in part mediated by TNF-α signal transduction via the TNFR1 receptor. The absence of TNF p55 receptor activity was most notable in the persistent, chronic phase of chemokine induction represented by a 28-day post-treatment analysis. MIP-1α mRNA and protein were significantly increased in both WT and TNFR1−/− mice at 24 h after silica exposure, suggesting an alternative stimulus for this chemokine, independent of TNFR1, at this time point. At 28 days after silica exposure, however, MIP-1α expression was markedly reduced in TNFR1−/− mice in comparison to a persistent elevation in WT mice. Consistent with independent regulation of the two chemokines, induction of MIP-2 mRNA and protein levels in lung tissue was significantly less in TNFR1−/− than in wild-type animals, even at 24 h after silica treatment. At 28 days, like MIP-1α, MIP-2 expression remained markedly elevated in WT mice but returned to near baseline in TNFR1−/− mice. Although MIP-2 may be increased by direct oxidant stress of silica exposure (Driscoll, 2000), this study suggests that at the relatively small dose of silica tested, MIP-2 expression is dependent on TNF-α receptor activity, partially in the acute phase, and more significantly in the chronic, persistent phase of silica-dependent chemokine induction.

The chemokines are largely regulated at a pretranslational level by the transcription factors NFκB and AP-1. Intratracheal silica was shown to induce NFκB transactivating function, in vivo, in bronchiolar epithelial cells and alveolar macrophages of NFκB-luciferase transgenic mice, in association with increased MCP-1 expression (Hubbard et al., 2002). In a recent study utilizing TNFR1−/− animals and an intratracheal silica dose of 200 mg/kg, NFκB but not AP-1 was stimulated by silica treatment independent of TNFR1−/− activity (Ortiz et al., 2001). TNF-α independent activation of NFκB may be responsible for the increase in MIP-1α expression observed in the first 24 h in both WT and TNFR1−/− mice in the current study. The return of MIP-1α mRNA to near basal levels by 28 days suggests that prolonged activation of NFκB, or of another factor regulating the chemokine, requires TNF function.

Neutrophil recruitment and protein leak into bronchoalveolar fluid was not significantly reduced in the TNFR1−/− mice, despite reduction in chemokine expression including MIP-2. Neutrophil activation and interstitial accumulation were not evaluated but PMN number and percentage in bronchoalveolar lavage were persistently elevated in TNFR1−/− as well as WT mice, even 28 days following the single silica intratracheal dose. In addition, a small but significant number of lymphocytes were detected in BAL of silica-exposed mice; this level also did not differ in TNFR1−/− mice. Consistent with these observations, dissociation between alveolitis as measured by BAL and TNF-α activity was previously suggested by Piguet et al. in studies utilizing recombinant soluble 55 kD TNF receptor to block TNF signal transduction in mice treated with
silica (Piguet and Vesin, 1994). Constant infusion of the soluble TNF receptor had no effect on inflammatory cells recovered by BAL, but it did prevent the increase in lung hydroxyproline content measured 15 days after silica treatment. Consistent with the current report in which the greatest effect of TNFRI ablation on chemokine expression appeared to be in the later phase of silica-induced chemokine expression, these observations support the hypothesis that TNFRI has a greater role in fibrosis than in acute alveolitis.

Factors responsible for the acute neutrophilic response and persistent cellular recruitment were not demonstrated in the present study. Prior studies in rats with anti-MIP-2 antibody infusion demonstrated marked reduction in neutrophil recruitment measured in BAL 24 h after silica treatment. In contrast, we demonstrate neutrophil recruitment despite reduced MIP-2 induction. The discrepancy between studies may be explained by differences in species and potentially more complete blockade of MIP-2 activity by antibodies than is caused in the TNFRI−/− mice. In our study, MIP-2 levels were increased by silica in the receptor knockout animals, though to a significantly lesser extent than in WT. It is possible that although the induction of the cytokines measured was attenuated in the absence of TNFRI, the small increase in expression that remained is adequate to stimulate PMN and lymphocyte recruitment. In addition, other molecules with potential neutrophil chemoattractant activity were not measured. ICAM-1 mRNA, measured by Northern blot, was not significantly elevated by this low dose of silica (data not shown). Modification of IL-1 and IL-1RA mRNA levels, in response to intratracheal silica, followed a pattern very similar to the chemokines discussed above, being less elevated in TNFRI−/− than in WT mice at both 24 h and 28 days (data not shown). In addition, in this work, only TNFRI p55 kD ablation was studied, leaving intact production of TNF-α and potential signal transduction via the TNFRI p75 kD receptor. It is possible that TNF-α, stimulated by silica, remains involved in neutrophil and lymphocyte recruitment to the lung via the TNFRI receptor. Future evaluation of low-dose silica exposure in double, p55 and p75 gene-ablated mice will address the importance of both receptors to silica-induced pulmonary inflammation.

In summary, a single intratracheal instillation of crystalline silica (50 μg) increased chemokine levels in mouse lung as represented by a 10- and 5-fold increase in MIP-2 and MIP-1α protein concentrations, respectively, 28 days after treatment. The chemokine response to this dose of silica was reduced in the absence of TNFRI, supporting the role of TNF-α, especially in prolonging host response to silica. Somewhat surprisingly, despite reduced chemokine gene expression, TNFRI function was not essential for recruitment of lavagable cells or intra-alveolar protein leak. The effect of loss of TNFRI function on collagen deposition and fibrosis could not be studied in this model, due to the mild nature of the response. Organizing granulomas were also not observed in this limited exposure. This model will be utilized for further studies to determine the effect of low-grade chemokine expression and neutrophil recruitment on subsequent silica exposure as well, as response to respiratory pathogens as may occur following human exposures.

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REFERENCES


