Clin Exp Immunol 2003;**131**:415–421

Silica accelerated systemic autoimmune disease in lupus-prone New Zealand mixed mice

J. M. BROWN*, A. J. ARCHER*, J. C. PFAU* & A. HOLIAN* **Center for Environmental Health Sciences, Department of Pharmaceutical Sciences, The University of Montana, Missoula,USA*

(Accepted for publication 16 December 2002)

SUMMARY

The genetic backgrounds of lupus-prone murine models are a valuable resource for studying the influence of environmental exposure on autoimmune diseases in sensitive populations. Epidemiological studies have shown associations between silica exposure and several autoimmune diseases, including scleroderma and systemic lupus erythematosus. To determine whether silica exposure can exacerbate systemic autoimmunity in genetically predisposed animals, New Zealand mixed mice were intranasally instilled twice with saline or saline suspensions of 1 mg silica or 500 μg TiO_2, a dose equivalent in surface area, and were evaluated with respect to health and immune status. Survival in silica exposed NZM mice was decreased compared to saline and TiO_2 exposed mice. Proteinuria levels were elevated in silica exposed mice. Levels of circulating immune complexes, autoantibodies to nuclear antigen (ANA), histone, and double stranded DNA were measured every two weeks by ELISA. Circulating immune complexes showed a trend towards an increased acceleration in levels in the silica exposed mice compared to saline and TiO_2 exposed mice. ANA levels were significantly higher in silica exposed animals compared to saline and TiO_2 exposed animals ($0{\cdot}237 \pm 0{\cdot}03$ *versus* $0{\cdot}140 \pm 0{\cdot}029$ and $0{\cdot}125 \pm 0{\cdot}03$, $P < 0{\cdot}05$) 16 weeks postexposure. Autoantibodies to histone were also significantly elevated after 16 weeks in silica exposed animals compared to saline and TiO_2 exposed animals ($0{\cdot}227 \pm 0{\cdot}03$ *versus* $0{\cdot}073 \pm 0{\cdot}015$ and $0{\cdot}05 \pm 0{\cdot}03$, $P < 0{\cdot}05$). In contrast, serum IgG levels were decreased in silica exposed NZM mice compared to the saline controls, however, IgM levels were unaffected. Lungs of the silica-exposed mice had increased inflammatory infiltrates as well as fibrotic lesions characterized by excess collagen deposition. Therefore, although NZM mice are susceptible to SLE, silica exposure significantly exacerbated the course of disease.

Keywords silica silicosis New Zealand mixed mouse systemic lupus erythematosus autoimmune disease

INTRODUCTION

Silica is ubiquitous in the environment as an abundant mineral found in rock, sand, and soil. Occupational silicosis results from acute or chronic exposures to high levels of silica dusts in many manufacturing and construction processes as well as mining operations. Silicosis leads to deceased pulmonary function and increased susceptibility to other diseases of the respiratory tract [1]. In addition, inhalation of silica has been associated with increased incidence of systemic autoimmune diseases, such as systemic lupus erythematosus (SLE) and scleroderma [2]. Human silicosis patients have exhibited increased autoantibodies, immunoglobulins and immune complexes [3]. However, the mechanisms leading to autoantibody production and the relationship to subsequent pathology have not been elucidated and these autoimmune reactions do not occur in all silica exposed individuals, suggesting a genetic component. Although some of the enhanced humoral response could be attributed to a nonspecific adjuvant effect, the tendency towards select autoimmune syndromes suggests a more specific effect.

A study by Steenland and Brown [4] demonstrated that 3000 gold miners exposed to silica had increased risk for autoimmune diseases, including SLE, rheumatoid arthritis, and scleroderma. Haustein and Anderegg [5] created a scleroderma registry from 1980 to 1997 and showed a 12-fold increase in scleroderma in males over the age of 40 with silicosis. Several other groups have reported an increased incidence of SLE associated with silicosis [6,7].

Correspondence: J. M. Brown, Center for Environmental Health Sciences, Department of Pharmaceutical Sciences, The University of Montana, 154 Skaggs Building, Missoula, Montana, 59812, USA.

E-mail: address: jaredb@selway.umt.edu

The present study was designed to test the hypothesis that silica exposure could exacerbate the development of autoimmune disease in a genetically susceptible murine model. Studies by Pollard *et al*. [8] have demonstrated that autoimmune prone mice are a valuable resource for studying the effects of environmental exposures on genetically susceptible populations. The New Zealand mixed (NZM) 2410 mouse model was selected to assess the effects of silica exposure and the exacerbation of predisposed autoimmune disease. NZM mice spontaneously develop features of SLE between 6 months and 1 years of age [9]. This model characteristically develops anti-dsDNA, anti-histone, and anti-nuclear antigen (ANA) autoantibodies as well as increasing levels of circulating immune complexes [9]. This strain also develops glomerulonephritis by one year of age in 80% of males and females [9]. The New Zealand mixed mouse was incorporated into this study due to the lower number of autoimmune susceptibility loci compared with murine models that have a full complement of SLE genes, such as the MRL/*lpr* or NZBxNZW F1, in which the severe autoimmune phenotype could mask any environmental insult [9].

The overall objective of the study was to test the hypothesis that inhaled silica, and not saline or a control particle (TiO_2), could exacerbate the natural progression of systemic autoimmune disease in SLE prone NZM mice. The disease course was measured by following the development of autoantibodies, serum immunoglobulins, immune complexes, proteinuria, and pulmonary fibrosis.

MATERIALS AND METHODS

Mice

Male and female New Zealand mixed (NZM 2410) mice were obtained from Taconic (Germantown, NY) and maintained in microisolation containers in accordance with the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources, National Research Council. The animal room is set on 12- h dark/light cycles with food and water provided *ad libitum*.

Treatment of mice

At six weeks of age, mice were instilled intranasally with either 30 μl saline ($n = 5$) or 30 μl saline suspensions of 1 mg crystalline silica ($n = 14$) or 500 μg TiO_2 ($n = 5$) as a control particle equivalent to silica in surface area. All mice received 2 instillations 2 weeks apart in order to represent several exposures over a period of time. Control and experimental groups were matched for the number of male and female mice. Silica was obtained from Pennsylvania Glass Sand Corp. (Pittsburgh, PA, USA). TiO_2 was obtained from Fisher Scientific (Denver, CO, USA). Mice were bled for sera before the first instillation and at 2-week intervals following instillations to monitor autoantibody levels. A second cohort of NZM mice was instilled with 30 μl saline ($n = 8$) or 30 μl of a saline suspension of 1 mg silica ($n = 8$) to use for histological examinations at 14 weeks. After 14 weeks, blood was collected for sera by cardiac puncture. The lungs and kidneys were removed for histology and the superficial cervical lymph nodes and spleens were weighed.

Detection of serum autoantibodies

ANA was detected by indirect immunofluoresence using HEp-2 cell slide kits (Immunoconcepts, Sacramento, CA, USA). Manufacturer's protocol was followed. ANA, anti-dsDNA, anti-histone antibodies and circulating immune complexes were detected by ELISA kits (Alpha Diagnostics, San Antonio, TX, USA). Sera were diluted 100-fold before assay and manufacturer's protocol was followed. Samples with a positive circulating immune complex level were determined by using a cut-off value as determined by the manufacturer. The reported values are mean optical density (OD) values from each treatment group.

Serum immunoglobulin quantification

Serum IgG and IgM levels were quantified by ELISA. 96 well Polysorp Nalge-Nunc ELISA plates (Fisher) were coated with 100 μl of 1 μg/ml goat anti-mouse kappa light chain antibody (Southern Biotechnology Associates, Birmingham, AL, USA) diluted in phosphate-buffered saline (PBS) and incubated overnight at 4°C. Plates were blocked with 200 μl PBS-1% bovine serum albumin (BSA) for 1 h at room temperature followed by 3 washes with PBS-0·1% Tween 20. Sera were diluted in PBS-0·1% Tween 20/1% BSA. Standard curves were generated using unlabelled IgG (Southern Biotechnology Associates) starting at 0·5 μg/ml and unlabelled IgM (Sigma Chemical, St. Louis, MO, USA) starting at 1 μg/ml. Diluted sera were added to wells and incubated in duplicate for 1 h at room temperature followed by 3 washes with PBS-0·1% Tween 20. HRP-conjugated goat anti-mouse IgG (Fc-fragment specific) (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and IgM (Jackson Laboratories) were diluted 2000-fold in PBS-0·1% Tween 20/1%BSA and incubated for 1 h at room temperature followed by 3 washes with PBS-0·1% Tween 20. 100 μl One Step TMB substrate (Zymed, San Francisco, CA, USA) was added and incubated for 15 min followed by the addition of 100 μl 2 N H_2SO_4 to stop the reaction. The optical density was read at 450 nm and serum immunoglobulin concentration was calculated by extrapolation from the linear portion of the standard curve. All samples were tested in duplicate.

Urinary protein

Proteinuria was measured by Chemstrip 2 GP test strips as described by the manufacturer (Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). Milligram protein per deciliter was measured between groups following the provided scale (0 = negative, trace, 1 + = 30 mg/dl, 2 + = 100 mg/dl, 3 + = 500 mg/dl).

Histology

Animals were given a lethal injection of sodium pentobarbital (5 mg i.p.) and the lungs were perfused with Histochoice fixative (Amresco Inc, Solon, IL, USA). Lungs and kidney were removed and routinely processed using an automated processor (ThermoShandon, Pittsburgh, PA, USA). The lungs and kidneys were embedded in paraffin wax and sectioned 5–7 μm thick, then collected on poly L-lysine coated slides (Sigma). Using an automated stainer (ThermoShandon) lung samples were stained with haematoxylin and eosin for general cellular morphology and Gomori Trichome for collagen deposition. Samples were blinded and examined by light microscopy. Kidney sections were immunohistochemically stained using an antibody for IgG immune complex and complement deposition. The kidney sections were boiled in a 0·01M sodium citrate buffer for 10 min followed by washes in distilled water and phosphate buffered saline. The kidney sections were then blocked with 4% fetal bovine serum in phosphate buffered saline. Goat anti-mouse IgG-FITC antibody (ICN Biomedicals, Irvine, CA, USA)

and a goat anti-mouse C3-FITC (ICN Biomedicals) were used for the detection of immune complexes and complement deposition. A goat anti-rat IgG antibody (ICN Biomedicals) was used as an isotype control. Samples were blinded and examined using a fluorescent microscope.

Statistical analysis

Differences between silica treated and saline or TiO_2 treated mice were assessed using analysis of variance (ANOVA) and unpaired *t*-test. All values are reported as mean ± SE; $P \leq 0{\cdot}05$ was considered significant.

RESULTS

Effects of silica and TiO_2 on mortality, proteinuria and circulating immune complexes in NZM mice

Mortality, proteinuria and immune complexes have been reported with silicosis [3], therefore these biomarkers were examined in NZM mice following instillation of saline or saline suspensions of TiO_2 or silica. Mortality in silica instilled NZM mice was exacerbated compared to saline and TiO_2 instilled animals (Fig. 1). Mortality in silica exposed NZM mice began around 10 weeks following instillation, while mortality in the saline and TiO_2 instilled mice didn't begin until 16 weeks following instillation. Within 22 weeks following instillation of silica, only 22% of the mice survived, while 60% of the mice instilled with saline or TiO_2 survived within the same time and continued to live until sacrificed at 9 months following exposure.

Although NZM mice have a rapid onset of glomerulonephritis with proteinuria levels greater than 500 mg/dl in both males and females [9], silica exposure exacerbated the development of proteinuria (Fig. 2). NZM mice instilled with silica developed proteinuria levels of 500 mg/dl within 10 weeks following instillation, while the saline instilled mice did not develop the same levels until 16 weeks following exposure. The TiO_2 instilled mice developed 500 mg/dl proteinuria levels 14 weeks following exposure. Sixteen weeks following exposure, 87·5% of the silica instilled NZM mice had 500 mg/dl levels of proteinuria, while only 33% of saline and TiO_2 had high proteinuria levels.

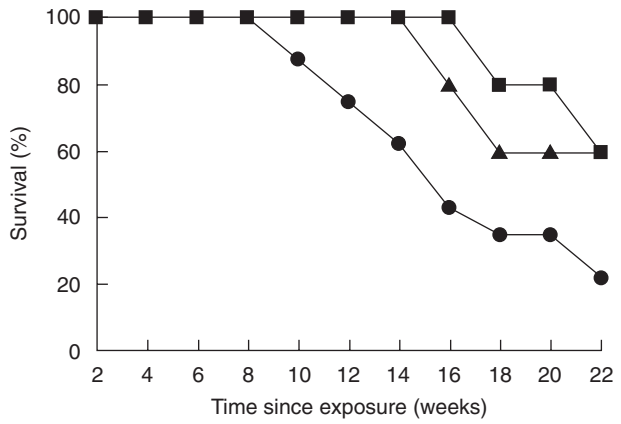

Fig. 1. Survival of saline (■) ($n = 5$), TiO_2 (▲) ($n = 5$) and silica (●) ($n = 14$) instilled NZM mice. Silica exposed NZM survival decreased more rapidly and to a greater extent than saline and TiO_2 exposed mice.

Levels of circulating immune complexes showed a trend towards accelerated elevation in silica instilled mice compared to saline and TiO_2 instilled mice (Fig. 3). The silica instilled NZM mice developed detectable levels of circulating immune complexes within 6 weeks following instillation, while it required 10 weeks postexposure for the TiO_2 exposed mice to develop detectable levels of circulating immune complexes. The saline exposed mice did not show detectable levels of circulating immune complexes until 12 weeks following instillation. However, within 14 weeks following exposure, all of the mice within the three groups developed circulating immune complexes. 16 weeks following exposure, fewer saline and TiO_2 exposed mice had detectable levels of circulating immune complexes, while all of the silica exposed mice continued to have detectable levels of

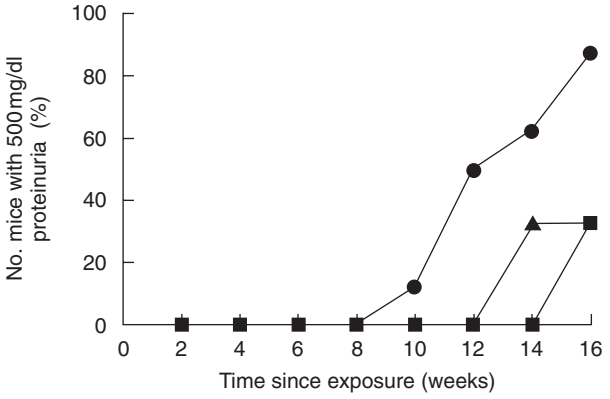

Fig. 2. Proteinuria levels greater than 500 mg/dl in saline (■) ($n = 5$), TiO_2 (▲) ($n = 5$) and silica (●) ($n = 14$) instilled NZM mice measured by Chemstrip 2GP proteinuria/glucose strips every two weeks. Silica exposed NZM mice developed high proteinuria levels earlier and in greater numbers than the saline or TiO_2 exposed mice.

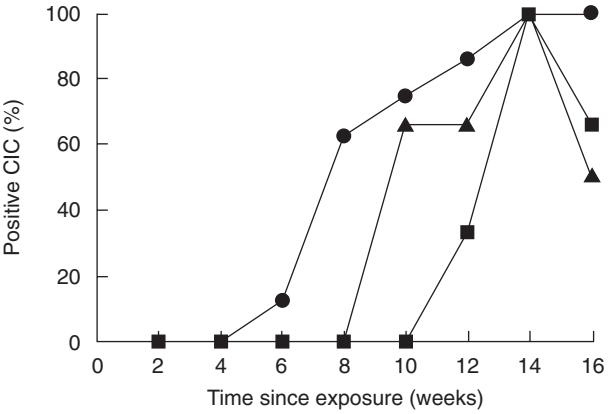

Fig. 3. Levels of circulating immune complexes (CIC) in serum from silica exposed NZM mice. Shown is the percentage of saline (■) ($n = 5$), TiO_2 (▲) ($n = 5$) and silica (●) ($n = 14$) exposed NZM mice that developed a positive level of CIC measured by ELISA every two weeks. Serum samples with an OD reading above a certain cut-off value that was set according to the manufacturer was considered to have positive levels of CIC.

circulating immune complexes. Taken together, these results demonstrate that silica exposure in NZM mice exacerbates mortality, possibly due to increases in proteinuria levels and circulating immune complexes.

Effects of silica and TiO_2 on autoantibody levels in NZM mice

Although NZM mice are susceptible to autoantibody formation, typically developing autoantibodies within six months of age [9], silica significantly elevated levels of serum ANA and anti-histone antibodies within 16 weeks following exposure compared to saline and TiO_2 (Fig. 4). ANA levels measured by ELISA were exacerbated in the silica-exposed animals compared to the saline and TiO_2 mice ($0{\cdot}237 \pm 0{\cdot}03$ *versus* $0{\cdot}140 \pm 0{\cdot}029$ and $0{\cdot}125 \pm 0{\cdot}03$, $P < 0{\cdot}05$, respectively) (Fig. 4a). ANA was also confirmed by HEp-2 fluorescent staining that consisted of a homogenous nuclear pattern (Data not shown). Anti-histone antibody levels were also elevated in silica treated mice compared to saline and TiO_2 treated animals ($0{\cdot}227 \pm 0{\cdot}03$ *versus* $0{\cdot}073 \pm 0{\cdot}015$ and $0{\cdot}05 \pm 0{\cdot}03$, $P < 0{\cdot}05$, respectively) (Fig. 4b). Autoantibodies to dsDNA were also examined, however, there were no differences between treatments (data not shown). These results demonstrate that silica exposure is able to elevate levels of autoantibody production in an autoimmune prone mouse model.

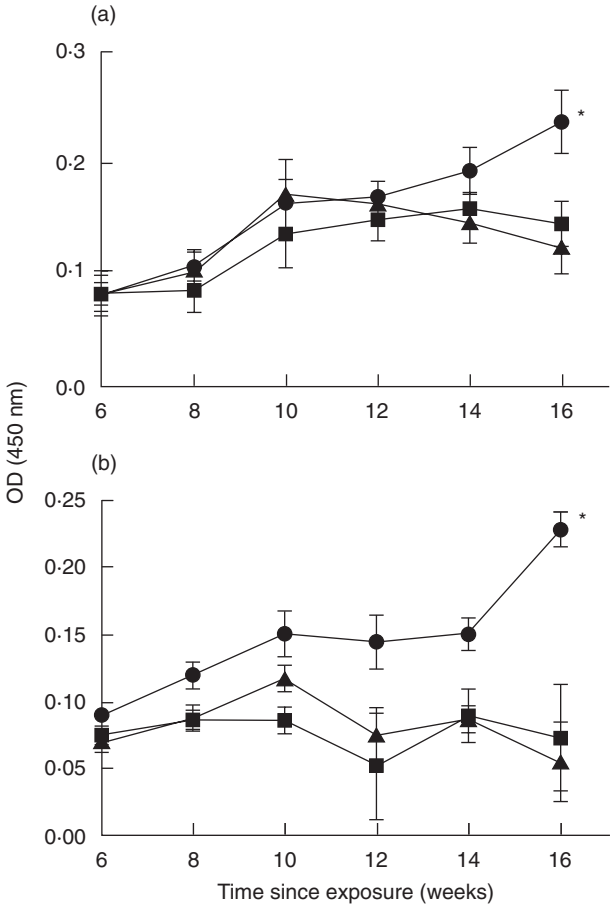

Fig. 4. Development of antinuclear antigen (ANA) and anti-histone autoantibodies in saline (■) ($n = 5$), TiO_2 (▲) ($n = 5$) and silica (●) ($n = 14$) exposed NZM mice measured by ELISA every two weeks. Silica exposure significantly elevated ANA levels 16 weeks following exposure compared to saline and TiO_2 (a). Levels of anti-histone autoantibodies 16 weeks following exposure were also elevated compared to saline and TiO_2 exposed mice (b). The values reported are mean values for each treatment group. The levels of ANA and anti-histone autoantibodies before 6 weeks were not different between treatments and therefore not shown. *($P \leq 0{\cdot}05$ using an unpaired *t*-test)

Effects of silica on serum immunoglobulin levels in NZM mice

Serum immunoglobulin levels have been reported to be increased in several models of silicosis [10,11], therefore IgG and IgM levels were examined in NZM mice following instillations of saline and silica. IgM and IgG levels were examined by ELISA using serum from saline and silica 14 week exposed mice that were sacrificed for lung histology. Fourteen weeks following silica exposure, IgG levels were decreased from an average of 3024 μg/ml in the saline exposed mice to 1638 μg/ml in the silica exposed mice (Fig. 5). However, the levels of IgG remained the same from 2 to 12 weeks (data not shown). There was no difference with IgM levels in the saline or silica exposed mice from 2 weeks to 14 weeks following exposure (data not shown). These results demonstrate that silica exposure specifically decreases IgG in the NZM mouse model contrary to increases in immunoglobulin levels in other models of silicosis.

Effects of silica on pathological changes in NZM mice

Saline and silica exposed NZM mice were sacrificed at 14 weeks following exposure to collect lung samples for histology and to examine lymph node and spleen weights. Lung sections were stained with H&E and Gomori's trichrome to examine the development of fibrosis. Histological examination of stained lung sections of silica exposed NZM mice revealed fibrotic lesions with excess collagen deposition, while the saline exposed mice had minimal collagen deposition (Fig. 6). H&E staining showed increased inflammatory infiltrates in the silica exposed NZM mice compared to the saline exposed mice (data not shown).

The kidneys of saline and silica exposed NZM mice were examined 14 weeks following exposure for the presence of immune complex and complement deposition. Immunohistochemical staining of kidneys revealed extensive IgG immune complex deposition within the glomeruli of silica exposed mice (Fig. 7). The saline exposed mice showed minimal staining for IgG immune complexes within the glomeruli. The kidneys were also

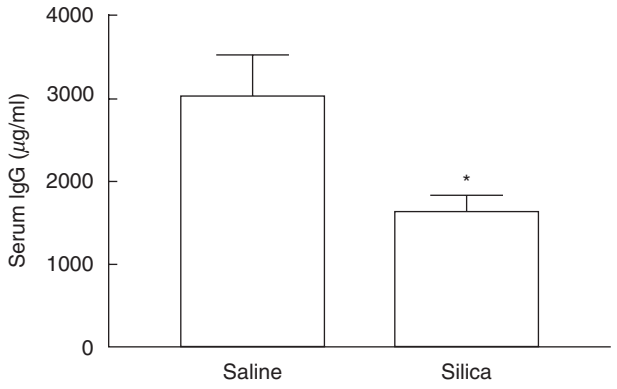

Fig. 5. Serum IgG levels in saline and silica exposed NZM mice 14 weeks following exposure measured by ELISA. IgG levels were significantly reduced in silica exposed mice ($n = 8$) compared to saline control mice ($n = 8$) *$P \leq 0{\cdot}05$ using an unpaired *t*-test.

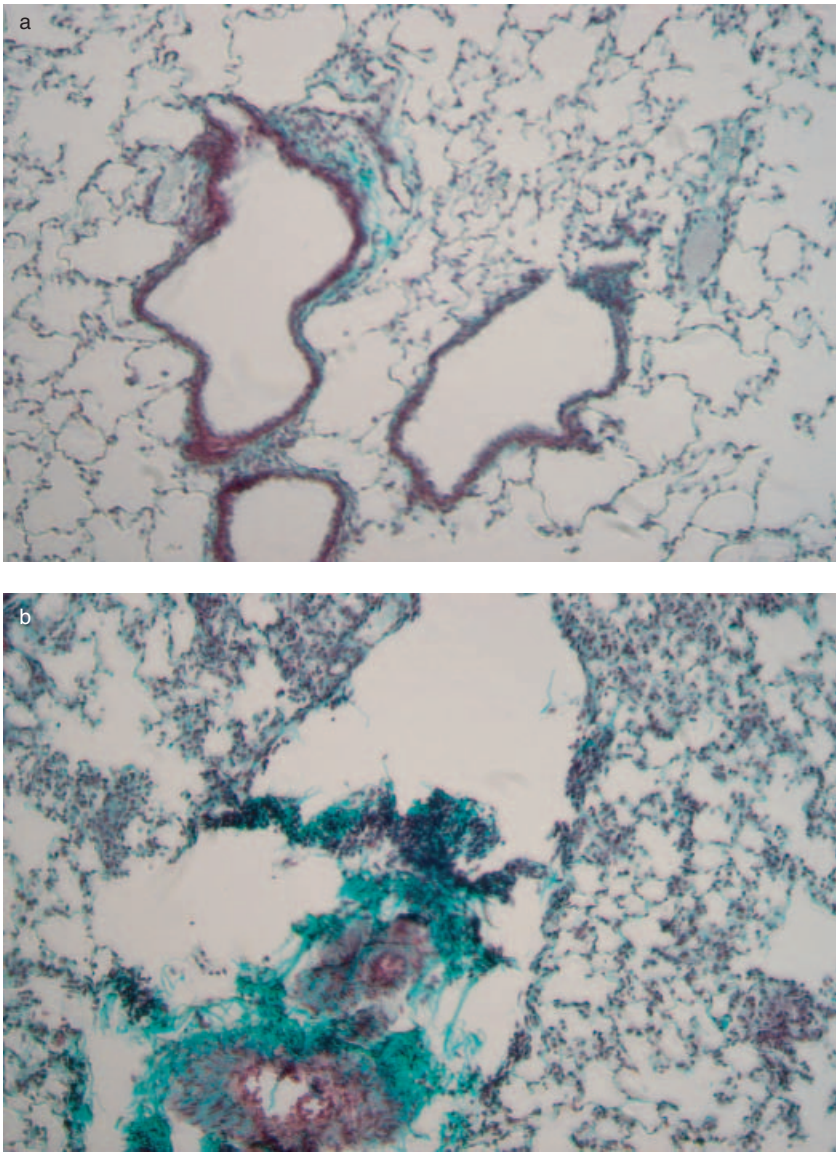

Fig. 6. Representative examples of Gomori trichrome staining of (a) a saline and (b) a silica exposed NZM mouse 14 weeks following exposure. The silica exposed mouse shows extensive fibrotic lesions and excess collagen deposition represented by the blue staining indicating development of silicosis. Saline exposed mice had very little collagen deposition.

stained for complement C3 deposition and showed similar patterns of staining within the glomerulus as the IgG immune complex staining (data not shown).

The superficial cervical lymph node was removed and weighed upon sacrifice of saline and silica treated NZM mice. A trend towards an increase in the average size of the lymph node in the silica treated mice (117·4 ± 33·3 g) compared to the saline treated mice (69·04 ± 20·7 g) was observed. However, there was no difference in spleen weight between the silica exposed (152 ± 15·7 g) and the saline exposed mice (146 ± 13·3 g). These results demonstrate that silica exposure in NZM mice is leading to development of silicosis with increased inflammation in the lung and increased immune complex and complement C3 deposition within the kidney.

DISCUSSION

The exacerbation of systemic autoimmune disease by silica exposure in New Zealand mixed mice identifies a potential model for the study of xenobiotic effects on autoimmune susceptible populations. This study demonstrates that silica is able to exacerbate the development of autoimmune disease in a genetically susceptible mouse model. The disease acceleration involved increases in mortality, proteinuria, autoantibody levels, circulating immune complexes, pulmonary fibrosis, immune complex deposition and complement C3 deposition within the kidney. The disease acceleration appears to be specific to silica and is not seen with a control particle, TiO_2, which has been reported to cause mild inflammation [12].

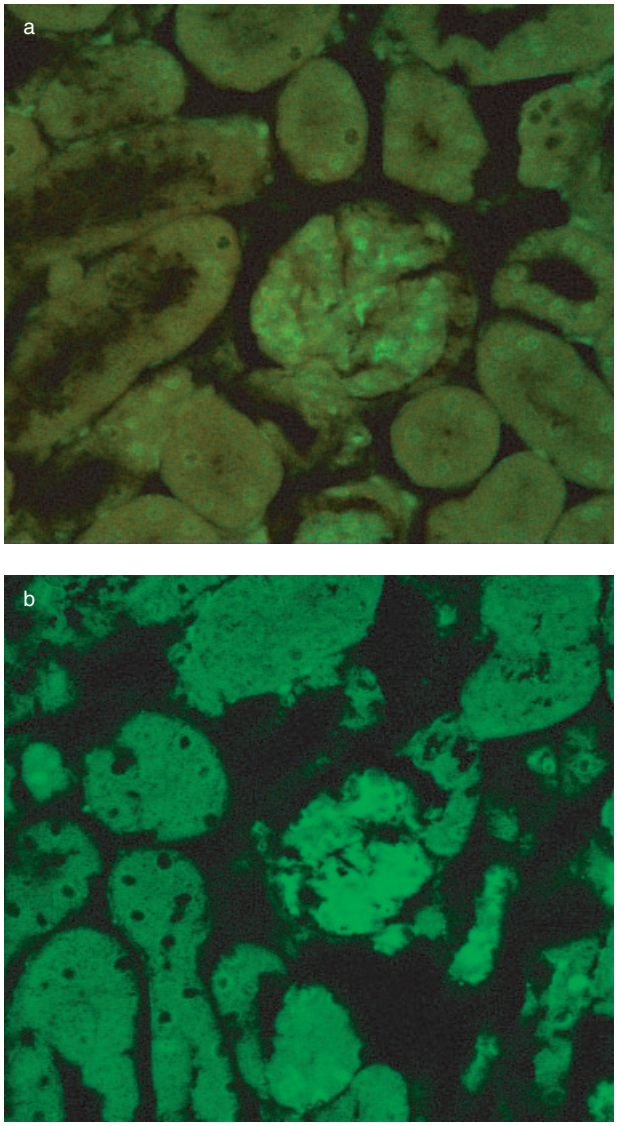

Fig. 7. Representative examples of immunohistochemical staining of kidney sections from (a) saline and (b) silica exposed NZM mice 14 weeks following exposure. A goat anti-mouse IgG-FITC antibody was used to stain immune complexes within the glomeruli of the kidney. The silica-exposed mice showed extensive immune complex deposition within the glomeruli of the kidney while the saline exposed mice had minimal staining for immune complexes. Magnification ×60.

The most pronounced response to silica exposure in the NZM mouse was a decrease in survival. The silica exposed NZM mice died significantly earlier than the saline or TiO_2 exposed mice, which corresponded with the development of proteinuria levels greater than 500 mg/dl. This suggests that mortality may have been associated with development of glomerulonephritis in the silica-exposed mice. Silica particles have been reported to accumulate in the kidney leading to renal fibrosis and localized inflammation [13]. However, our model indicates that circulating autoantibodies are deposited within the kidney resulting in an immune complex mediated glomerulonephritis. This is supported by the rapid development of high titres of circulating immune complexes, proteinuria and immune complex deposition within the kidneys of the silica-exposed mice.

Possible mechanisms of silica induced autoimmune disease may involve the alveolar macrophage, the primary immune cell of the lung that is the first line of defense and the primary cell involved in the clearance of apoptotic cells within the lung. It has previously been reported that phagocytosis of silica leads to a caspase dependent apoptosis in human alveolar macrophages [14]. Silica induced apoptosis of alveolar macrophages leads to release and phagocytosis of silica by other alveolar macrophages, producing a cyclical process of inflammation and cell death [15]. This constant inflammation and cellular death may provide excess antigen that is being presented to the immune system, thereby breaking immune tolerance.

In this study, autoantibodies to nuclear antigen, including dsDNA and histone were examined as biomarkers of disease acceleration because NZM mice have been reported to develop high titres of these within 6 months to 1 years of age [9]. Silica exposure significantly elevated ANA levels and autoantibodies to histone, but had little effect on dsDNA autoantibody levels. The increase in titres of these autoantibodies may be the result of silica induced apoptosis leading to excess apoptotic material being presented to the immune system.

Several studies have examined immunoglobulin responses to experimental silicosis. These studies have reported that silicosis is associated with elevated IgG and IgM levels in the blood of Fisher rats [10]. Another study examined IgG subclass responses in experimental silicosis, also in Fisher rats, and reported increased IgG-secreting spot-forming cells of all IgG subclasses in lung associated lymph nodes [11]. However, our results with NZM mice show a decrease in IgG levels 14 weeks following silica exposure and no change in IgM levels from 2 to 14 weeks postexposure. In spite of the decrease in total IgG levels there was an increase in autoantibodies of the IgG class. This suggests silica may be decreasing total IgG, but a higher percentage of the immunoglobulin appears to be autoreactive. The decrease in IgG levels may be in part explained by the overall health of the mice. The majority of the silica exposed mice died within 16 weeks of exposure and the IgG levels were measured 14 weeks following silica exposure. Severe weight loss occurred one to two weeks before the silica exposed mice died. It is possible the immune system was compromised at this point and unable to produce normal levels of IgG. However, a trend towards an increase in the size of the superficial cervical lymph nodes in the silica-exposed mice was observed, suggesting increased immune activity.

The silica exposed NZM mice developed pulmonary fibrosis, visualized with Gomori trichrome stain for collagen deposition, while the saline exposed mice did not develop fibrosis. However, it is unknown whether the fibrosis is due to direct action of the silica or could be exacerbated by the increase in autoantibodies and immune complexes. Future studies will examine if deposition of immune complexes occurs in these silicotic lungs. H&E staining further showed significant infiltration of inflammatory cells into the lungs of the silica treated NZM mice. Future studies will examine which inflammatory cells are being recruited to the lung and the role they may play in the development of autoimmune disease. Staining of kidney sections from silica exposed NZM mice for immune complex and complement C3 deposition revealed extensive immune complex mediated glomerulonephritis.

In summary, silica exposure in the NZM 2410 mouse model of SLE appears to accelerate the development of autoimmune

disease. This exacerbation of disease involved increased mortality, proteinuria, autoantibodies, immune complexes, pulmonary fibrosis and immune complex mediated glomerulonephritis. The NZM mouse represents a good model to further study the mechanisms of xenobiotic induced autoimmune diseases in a genetically susceptible population.

ACKNOWLEDGEMENTS

This work was supported by NIH grant ES-04084, NIH NRSA grant ES-11249 and an American Foundation for Pharmaceutical Education (AFPE) Fellowship.

REFERENCES

1 American Thoracic Society Committee of the Scientific Assembly on Environmental and Occupational Health. Adverse effects of crystalline silica exposure. Am J Respir Crit Care Med 1997; **155**:761–8.

2 Parks CG, Conrad K, Cooper GS. Occupational Exposure to Crystalline Silica and Autoimmune Disease. Environ Health Perspect 1999; **107**:793–802.

3 Doll NJ, Stankus RP, Hughes J, Weill H, Gupta RC, Rodriguez M, Jones RN, Alspaugh MA, Salvaggio JE. Immune complexes and autoantibodies in silicosis. J Allergy Clin Immunol 1981; **68**:281–5.

4 Steenland K, Brown D. Mortality study of gold miners exposed to silica and nonasbestiform amphibole minerals: an update with 14 more years of follow-up. Am J Ind Med 1995; **27**:217–29.

5 Haustein UF, Anderegg U. Silica induced scleroderma – clinical and experimental aspects. J Rheumatol 1998; **25**:1917–26.

6 Brown L, Gridley MG, Olsen JH, Mellemkjaer L, Linet MS, Fraumeni Jr JF. Cancer risk and mortality patterns among silicotic men in Sweden and Denmark. J Occup Environ Med 1997; **39**:633–8.

7 Wilke RAS, Salisbury E, Abdel-Rahman, PC. Brazy Lupus-like autoimmune disease associated with silicosis. Nephrol Dial Transplant 1996; **11**:1835–8.

8 Pollard KM, Pearson DL, Hultman P, Hildebrandt B, Kono DH. Lupus-prone mice as models to study xenobiotic-induced acceleration of systemic autoimmunity. Environ Health Perspect 1999; **107**:729–35.

9 Rudofsky UH, Lawrence DA. New Zealand mixed mice: a genetic systemic lupus erythematosus model for assessing environmental effects. Environ Health Perspect 1999; **107**:713–21.

10 Huang SH, Hubbs AF, Stanley CF, Vallyathan V, Schnabel PC, Rojanasakul Y, Ma JK, Banks DE, Weissman DN. Immunoglobulin responses to experimental silicosis. Toxicol Sci 2001; **59**:108–17.

11 Weissman DN, Hubbs AF, Huang SH, Stanley CF, Rojanasakul Y, Ma JK. IgG subclass responses in experimental silicosis. J Environ Pathol Toxicol Oncol 2001; **20**:67–74.

12 Yuen IS, Hartsky MA, Snajdr SI, Warheit DB. Time course of chemotactic factor generation and neutrophil recruitment in the lungs of dust-exposed rats. Am J Respir Cell Mol Biol 1996; **15**:268–74.

13 Slavin RE, Swedo JL, Brandes D, Gonzalez-Vitale JC, Osornio-Vargas A. Extrapulmonary silicosis. a clinical, morphologic, and ultrastructural study. Hum Pathol 1985; **16**:393–412.

14 Iyer R, Hamilton RF, Li L, Holian A. Silica-induced apoptosis mediated via scavenger receptor in human alveolar macrophages. Toxicol Appl Pharmacol 1996; **141**:84–92.

15 Cooper GS, Miller FW, Germolec DR. Occupational exposures and autoimmune diseases. Int Immunopharmacol 2002; **2**:303–13.