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Effects of In Vitro Exposure to Silica on Bioactive Mediator Release by Alveolar Macrophages

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=ABSTRACT=

Alveolar macrophages play a pivotal role in the pathogenesis of silicosis since the macrophages may release a wide variety of toxic and inflammatory mediators as well as mitogenic growth factors. In the present study, the effects of *in vitro* exposure to silica on release of various mediators such as reactive oxygen species, platelet activating factor(PAF), and interleukin-1 (IL-1) by alveolar macrophages were examined. First, hydrogen peroxide release from alveolar macrophages was monitored by measuring the change in fluorescence of scopoletin in the absence or presence of graded concentration of silica. Significantly enhanced release of hydrogen peroxide was observed at 0.5 mg/ml and above. A maximal enhancement of 10 fold above control was observed at 5 mg/ml silica. Similarly, *in vitro* exposure to silica also significantly stimulated the generation of chemiluminescence from alveolar macrophages at 0.5 mg/ml and above with a maximal enhancement of 8 fold at 5 mg/ml silica. Second, PAF release from alveolar macrophages after 30 min incubation at 37℃ in absence or presence of zymosan and silica was determined by measuring ^{3}H-serotonin release ability of the conditioned macrophage supernates from platelets. 5 mg/ml zymosan as a positive control for the PAF assay increased PAF release by 19 % of total serotonin release. Furthermore, silica also resulted in significant enhancement of the PAF release compared with that in unstimulated (control) cells, i.e., 17.7±5.8% and 24.0±4.9% of total serotonin release at 5 mg/ml and 10 mg/ml silica, respectively, which represents the release of nanomole levels of PAF. Lastly, IL-1 production by alveolar macrophages was analysed following their stimulation with lipopolysaccharide (LPS) and silica by their capacity to stimulate thymocyte proliferation. 10 μg/ml LPS resulted in an 11 fold increase in IL-1 production. In comparison, 50 μg/ml silica resulted in a 4 fold increase in IL-1 release. These data indicate that *in vitro* exposure of alveolar macrophages to silica activates the release of various bioactive mediators such as reactive oxygen species, PAF and IL-1 which thus contribute to amplification of inflammatory reactions and regulation of fibrotic responses by the lung after inhalation of silica.

Key Words: Alveolar macrophages, Silica exposure, Reactive oxygen species, Platelet activating factor, Interleukin-1

INTRODUCTION

Silicosis is an inflammatory and fibrotic lung disease caused by inhalation and deposition of dust containing silicon dioxide or silica (SiO_2) in various forms. Several mechanisms for the development of silicosis have been proposed. One of these involves direct tissue damage by silica.

Recently, the presence of silicon-based radicals on the surface of freshly ground silica particles has been demonstrated and these silicon-based radicals resulted directly in lipid peroxidation and membrane damage to lung cells (Farber, 1982; Maridonneau et al, 1982; Vallyathan et al, 1988).

Another proposed mechanism for the development of silicosis suggests that interactions between silica and alveolar macrophages play pivotal roles in the pathogenesis of silicosis (Heppleston & Styles, 1967; Bateman et al, 1982). Inhaled silica comes in contact with or is phagocytized by alveolar macrophages, throughout the time the particles remain in the lung. The macrophages are stimulated to secrete reactive forms of oxygen, proteolytic enzymes, and lysosomal enzymes capable of damaging lung parenchyma (deShazo, 1982; Heppleston, 1982; Davis, 1986). At the same time, activated alveolar macrophages may release a wide variety of inflammation mediators such as chemotactic factors (Lugano et al, 1981; Reynolds, 1983), complement C_{5a} (Reynolds, 1983), leukotriene B-4 (Martin et al, 1984), prostaglandin E_2 (Hsueh et al, 1980) and platelet activating factor (Arnoux, 1980; Rylander & Beijer, 1987). These mediators would act to attract neutrophils into pulmonary airways. Finally, activated macrophages and recruited neutrophils would secrete increased amount of reactive products resulting in a cycle of inflammation, significant tissue damage, and fibrosis. Recently, platelet activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glycerol-3-phosphorylcholine) has emerged as a potentially crucial mediator in pulmonary inflammation, fibrosis, edema, emphysema, bronchoconstriction and bronchial asthma since PAF is produced by various cell types including alveolar macrophages (Rylander & Beijer, 1987; Arnoux, 1980), neutrophils(Betz & Henson, 1980), and basophils (O'Donnel et al, 1978) exposed to allergic or nonallergic stimulants.

Silica-activated alveolar macrophages have also been hypothesized to stimulate fibroblast proliferation, and enhance collagen production through the release of growth factors, i.e., interleukin-1, platelet derived growth factor, alveolar macrophage-derived growth factor, or fibronectin (Bitterman et al, 1982; Schmidt et al, 1984). Interleukin-1 (IL-1) is a major macrophage-derived cytokine that has been shown to exhibit multiple biological effects on a variety of target cells. It is known to cause leukocyte (Sauder et al, 1984) and endothelial cell activation (Movat et al, 1987), and to affect the synthesis and maintenance of the extracellular matrix by stimulation of fibroblast proliferation (Prostlethwaite et al, 1984; Schmidt et al, 1984), synthesis of collagens (Goldring & Krane, 1986) and glucosaminoglycans (Bronson et al, 1987) and prostaglandin E_2 production (Dayer et al, 1979). In addition, IL-1 plays an important role in the initiation of an immune response through its direct activation of lymphocytes and stimulation of the production of other cytokines, such as interferons and hemopoietic colony-stimulating factors needed for the expression of an immune response (Dinarello, 1989).

The objectives of the present investigation are to determine whether *in vitro* exposure to silica induces release of various bioactive mediators such as reactive oxygen species, PAF, and IL-1 from alveolar macrophages.

METHODS

Measurement of the release of reactive forms of oxygens

Cell preparation: Alveolar macrophages were harvested from pathogen-free male Sprague-Dawley rats (250~300 g) by bronchoalveolar lavage with Ca^{++}, Mg^{++} free Hank's balanced salt solution (145 mM NaCl, 5 mM KCl, 1.9 mM NaH_2PO_4 and 5.5 mM glucose, pH=7.4). Macro-

phages were centrifuged at 500 g for 5min, washed, and resuspended in HEPES-buffered solution (145 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 10 mM HEPES and 5.5 mM glucose, pH=7.4). Cell numbers and purity were measured using an electronic cell counter with a cell sizing attachment (Castranova et al, 1979).

Silica-induced activation of the respiratory burst in alveolar macrophages was monitored by measuring hydrogen peroxide release and chemiluminescence generation

H_2O_2 release: Hydrogen peroxide release was measured fluorometrically at an excitation wavelength of 330 nm and an emission wavelength of 460 nm by monitoring the oxidation of scopoletin (1.2 μM) in the presence of horseradish peroxidase type IX (6.6 units/ml) (Van Scott et al, 1984). Briefly, alveolar macrophages (4×10^6) were preincubated at 37℃ in 3 ml of HEPES-buffered medium for 10 min. After preincubation, scopoletin (1.2 μM) and type IX horseradish peroxidase (6.6 units/ml) were added and fluorescence at rest or in the presence of silica (0.005~10 mg/ml; Min-U-Sil, 99% < 5 μm, 98.5% silica). In this assay, the rate of hydrogen peroxide secretion was expressed as nmol/4×10^6 cells/3 min using a standard curve.

Chemiluminescence generation: The ability of silica to activate alveolar macrophages was also determined measuring cellular chemiluminescence using a liquid scintillation counter (Model 2002, Packard Instrument Co., IL, U.S.A.) as described by Jones et al (1981). Briefly, alveolar macrophages (4×10^6) were preincubated at 37℃ for 10 min in 5ml of HEPES-buffered medium containing luminol (10^{-5}M). After preincubation, chemiluminescence generation was monitored for 20 min at rest or in the presence of silica (0.05~10mg/ml), and expressed as total light, i.e., integrated counts, generated over 20 min measured gravimetrically.

Measurement of the release of PAF

The reasons why rabbits were used in stead of rats in order to determine PAF release by alveolar macrophages after *in vitro* exposure to silica were as follow. First, rat platelets do not possess high affinity binding sites, whereas these have been found in rabbit platelets (Godfroid & Braquet, 1986). Second, large blood volume(above 50 ml) should be collected to obtain sufficient number of platelets to accomplish the PAF assay. Therefore, rats are not appropriate animal to test the PAF release.

Cell preparation: Alveolar macrophages were harvested from New Zealand white rabbits (2 kg) by bronchoalveolar lavage as described previously. To obtain rabbit platelets, whole blood was collected by cardiac puncture with siliconized 18 gauge needle attached to a 50ml syringe containing 8 ml of 3.2% sodium citrate. Platelets were partially purified by the method of Ardlie et al (1970). Platelet-rich plasma was obtained by centrifugation of blood at 120 g for 15 min at room temperature. The supernate was collected and platelets pelleted at 1100 g 15 min at room temperature. The pelleted platelets were washed once in Tyrode's solution A (136.9 mM NaCl, 2.63 mM KCl, 2.2 mM $MgCl_2$, 12.1 mM $NaHCO_3$, 5.6 mM glucose, 2.5 g/l gelatin, and 0.038 g/l EGTA; pH=6.5). Platelets were then resuspended in 3 ml of Tyrode's solution B (Tyrode's A without EGTA) and incubated for 20 min at 37℃ with 0.75 μci of ^{3}H-serotonin/ml (30.4 ci/mM specific activity; New England Nuclear, MA, U.S.A.). After loading, platelets were washed once with Tyrode's solution A, once with Tyrode's solution B, and resuspended in 2 ml of Tyrode's solution (Tyrode B plus 1.3 mM $CaCl_2$; pH=7.3).

PAF release: Rabbit alveolar macrophages (1×10^7) were suspended in 1 ml RPMI-1640 con-

taining 3×10^{-3} HEPES and 2.5 g/l BSA and incubated for 30 min at 37°C in the absence or presence of zymosan (5 mg/ml), and silica (5 mg/ml, 10 mg/ml). After incubation, suspensions were centrifuged for 5 min at 560 g and 22℃. PAF content of alveolar macrophages supernates was estimated from the ability of these samples to induce serotonin release from platelets (O'Donnell et al, 1978). Briefly, 100 μl of the supernate from alveolar macrophage suspensions was added to an 100 μl suspension of platelets loaded with ^{3}H-serotonin, and incubated for 10 min at 37°C. The reaction was stopped by the addition of 50 μl of 8mM EDTA and centrifuged at 2000 g for 5 min. 100 μl of the supernate was added to 10ml of aquasol and ^{3}H-serotonin monitored in a liquid scintillation counter (Model 2425, Packard Tri Carb L.Q. Spectrometer, IL, U.S.A.). The total intracellular serotonin available for release was determined by measuring ^{3}H-serotonin in samples of platelet suspensions treated with 100 μl of 0.2% Triton ×100. All test values were corrected for resting serotonin release by subtraction of dPM values from control samples containing only cells and buffer. Release values then were calculated as percentage of total cellular serotonin and converted to nmoles of PAF released using PAF standard curves.

Measurement of the release of IL-1

Alveolar macrophage cultures and supernate preparation: Alveolar macrophages were obtained from male Sprague-Dawley CD rats (200~250 g) by bronchoalveolar lavage as previously. Lavage cells were washed with 50ml of the same buffer solution and resuspended in RPMI-1640 media (Whittaker, U.S.A.) containing 2mM glutamine, 100 units/ml mycostatin, and 10% heat-inactivated fetal calf serum. Cell number and purity of macrophage preparation were determined using an electronic cell counter equipped with a cell sizing attachment. Aliquots of 1ml containing 1×10^6 cells were added to 24 well plates (Costar, MA, U.S.A.) and incubated at 37℃ in a humidified atmosphere of 5% CO_2 for 2h. The nonadherent cells were then removed with two 1ml washes of the RPMI media. The adherent cells were further incubated in 1ml of the RPMI media containing 10 μg/ml LPS from Escherichia coli (Difco, MI, U.S.A.), and 50 μg/ml silica dust. After incubating the cell cultures for 20 h, the supernates were collected, filtered through a 0.22 μm Miller filter (Millipore, MA, U.S.A.), and frozen supernates were thawed when the thymocyte proliferation assay was performed.

Thymocyte proliferation assay for IL-1: IL-1 production by silica-exposed alveolar macrophages were determined by their capacity to stimulate thymocyte proliferation as modified the method of Lackman et al (1980). Briefly, thymocytes were obtained from male CD-1 mice (6~10 weeks of age) and suspended in RPMI-1640 media with 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 100units/ml mycostatin, 10% heat-inactivated fetal calf serum, and 2×10^{-5} M mercaptoethanol. Cells were counted using an electronic cell counter and adjusted to a concentration of 10×10^6 cells/ml. An aliquot of 100 μl of the macrophage-conditioned supernates was added in quadruplicate to 96-well microculture plates, and 100 μl of thymocyte suspension was placed in each well. After 48h incubation at 37℃ in 5% CO_2, the cultures were pulsed for 4-6 h with ^{3}H-thymidine (1.0 μCi/well, activity; 2.0 Ci/mmol, Dupont NEN Products, MA, U.S.A.), and harvested using a PHD cell harvester (Cambridge Technology, Inc., ME, U.S.A.). The radioactivity in the collecting glass filter disks was measured using a liquid scintillation counter (1214 Rackbeta, Wallac, Finland). The levels of IL-1 activity in the tested macrophage supernates were expressed as

counts per minutes and then calculated as a percentage of the unstimulated control sample response.

Statistics

Data were expressed as means ± standard errors of n experiments. Statistical significance was determined using a Student's t test with significance set at $P < 0.05$.

RESULTS

The present study attempted to determine whether *in vitro* exposure to silica activated the respiratory burst in alveolar macrophages. End points measured were hydrogen peroxide release and chemiluminescence generation.

Hydrogen peroxide release from alveolar macrophages was monitored by measuring the change in fluorescence of scopoletin while the cells were resting and while they were being stimulated by silica (5 mg/ml) (Fig. 1). The slope of the fluorescence change is proportional to the rate of hydrogen peroxide release. These data indicate that unstimulated cells did not exhibit hydrogen peroxide release activity. In contrast, *in vitro* exposure of alveolar macrophages to silica substantially activated hydrogen peroxide release. The

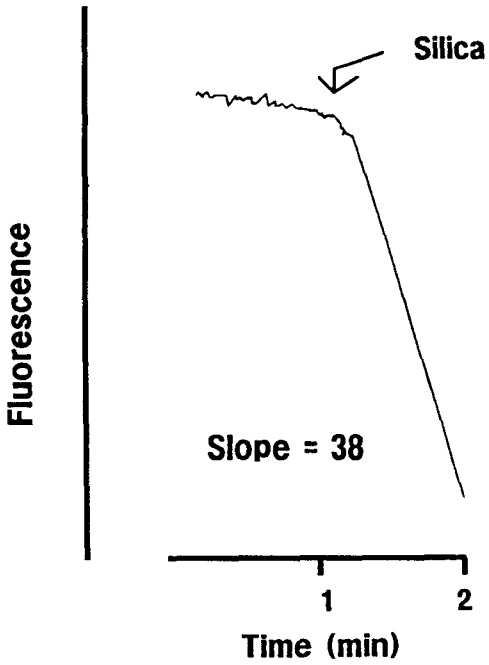

Fig. 1. Silica-induced hydrogen peroxide release. Alveolar macrophages (4×10^6 cells) were exposed in vitro to silica (5 mg/ml) and the change in fluorescence of scopoletin in the presence of horseradish peroxidase was monitored at 37℃. The slope of the fluorescence change is proportional to the rate of hydrogen peroxide release. Values are from a single experiment which is representative of results from six separate experiments.

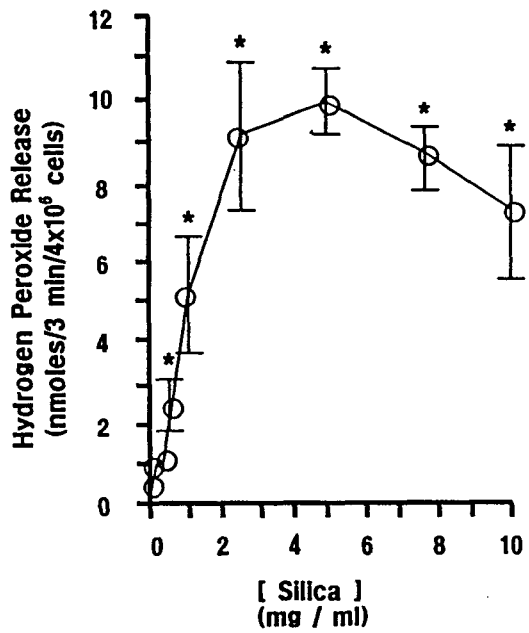

Fig. 2. Silica-induced hydrogen peroxide release. Alveolar macrophages (4×10^6 cells) were exposed in vitro to silica (0.05~10 mg/ml) and hydrogen peroxide secretion measured at 37℃. Values represent means ± standard errors for six separate experiments for each exposure level. Asterisks indicate significant increases above rest at $p < 0.05$ using the Student's t-test.

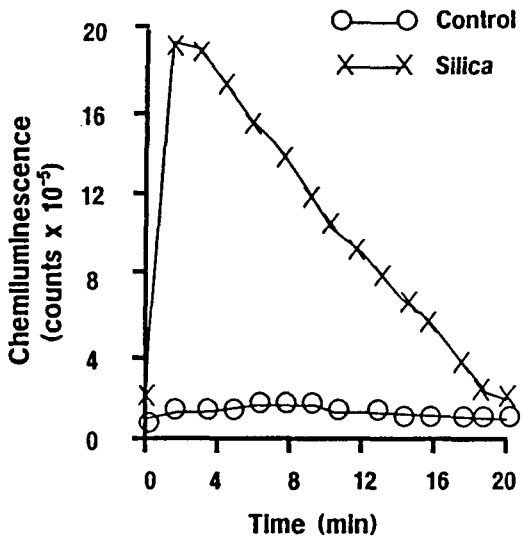

Fig. 3. Silica-induced chemiluminescence. Alveolar macrophages (4×10^6) were exposed in vitro to silica (2.5 mg/ml) and generation of chemiluminescence measured at 37℃. Values are from a single experiment which is representative of results from five separate experiments.

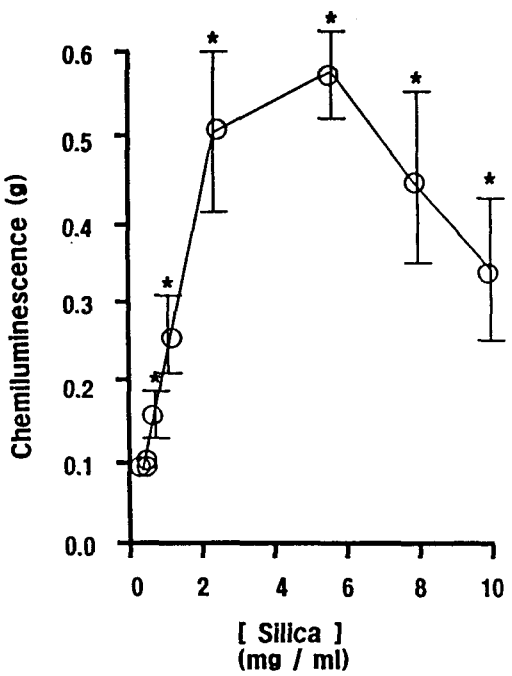

Fig. 4. Silica-induced chemiluminescence. Alveolar macrophages (4×10^6) were exposed in vitro to silica (0.05~10 mg/ml) and chemiluminescence measured at 37℃. Values represent means ± standard errors from five separate experiments for each exposure level. Asterisks indicate significant increases above rest at $p < 0.05$ using the Student's t-test.

dose-response relationship for silica-activated hydrogen peroxide with alveolar macrophages is given in Fig. 2. Statistically significant ($p < 0.05$) elevations were noted at silica exposure 0.5 mg/ml and above. A maximal enhancement of 10 fold above control was observed at 5 mg/ml silica. Above this dose of silica, hydrogen peroxide release activity began to decline slightly. *In vitro* exposure to silica also stimulated the generation of chemiluminescence by alveolar macrophages (Fig. 3). In unstimulated (control) cells a low level of chemiluminescence was generated. Chemiluminescence was enhanced in silica-treated cells reaching a peak 1.5 min after exposure to 2.5 mg/ml silica. The data indicate that *in vitro* exposure to silica activated chemiluminescence by 7 fold above control. Chemiluminescence was significantly enhanced in a dose related manner at silica exposure of 0.5 mg/ml and above (Fig. 4). A maximal stimulation of 8 fold was observed at 5 mg/ml silica. Above this dose of silica, the activation began to decline.

In vitro exposure of alveolar macrophages to silica resulted in secretion of PAF (Fig. 5). As expected little or no PAF release was observed with unstimulated cells while *in vitro* exposure of alveolar macrophages to zymosan (5 mg/ml) induced PAF release and was used as a positive control for the PAF assay. Furthermore, exposure of alveolar macrophages to silica also resulted in significant increase in the PAF secretion compared with that in unstimulated (control) cells, i.e., 17.7±5.8%,

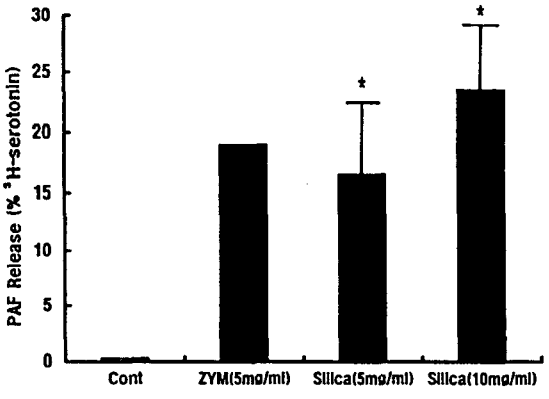

Fig. 5. Silica-induced PAF release.
Alveolar macrophages(1×10^7) were exposed in vitro to zymosan (5 mg/ml) and silica (5 mg/ml, 10 mg/ml) for 30 min at 37℃. PAF release was monitored by measuring ^{3}H-serotonin release from platelets. Data are expressed as % of total platelet-associated serotonin after subtractions of dPM values from control sample containing nonstimulated cells. Values represent means ± standard errors of four separate experiments. Asterisks indicate significant increases from control at $p < 0.05$ using the Student's t-test.

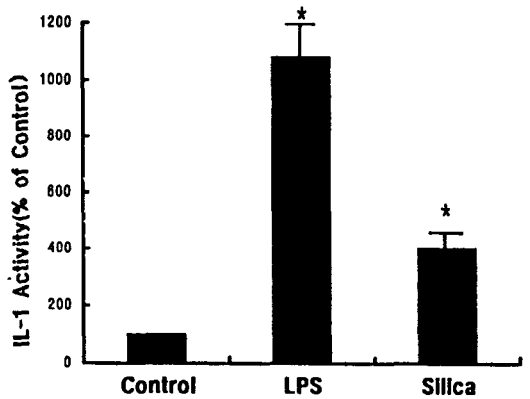

Fig. 6. Silica-induced IL-1 release.
Alveolar macrophages were cultured in the presence of LPS (10 μg/ml) and silica (50 μg/ml) for 20 h at 37℃, and IL-1 production by alveolar macrophages was monitored by measuring thymocyte incorporation of ^{3}H-thymidine by macrophage supernates. Data are expressed as percentage increase over control levels of nonstimulated cells. Values represent means ± standard errors of three experiments. Asterisks indicate significant increases from control at $p < 0.05$ using the Student's t-test.

and 24.0±4.9% of total serotonin release at 5 mg/ml and 10 mg/ml silica, respectively, which represent the release of nanomole levels of PAF using a standard curve of % ^{3}H-serotonin release from platelets vs concentration of PAF.

Fig. 6 shows the levels of IL-1 activity, as a percentage of the unstimulated (control) samples, in supernates from the alveolar macrophages treated with LPS and silica. LPS (10 μg/ml) resulted in an 11 fold increase in IL-1 production, and was used as a positive control for the thymocyte proliferation assay. In comparison, silica (50 μg/ml) resulted in a 4 fold stimulation of IL-1 release. Addition of the stimulants (LPS, silica) to thymocyte cultures did not show any effect on the thymocyte incorporation of ^{3}H-thymidine.

DISCUSSION

While the mechanism of pulmonary silicosis is still not clear, many studies have suggested that the fibrotic sequel is related to inflammatory process produced by macrophage activation, involving the release of oxygen-derived free radicals (i.e., superoxide anion, hydrogen peroxide, hydroxyl radical and singlet oxygen), and various inflammatory and fibrogenic mediators.

In the present study, *in vitro* exposure to silica activates the respiratory burst in alveolar macrophages with dose-related pattern, i.e., hydrogen peroxide release (Fig. 2) and chemiluminescence generation (Fig. 4). Maximal stimulation of H_2O_2 secretion and chemiluminescence was observed at

the same exposure dose of silica (5 mg/ml). Above this dose, the stimulation declined suggesting cytotoxic effects of silica on alveolar macrophages.

These results are consistent with findings with *in vivo* exposure of silica-induced activation of alveolar macrophages reported by Castranova et al (1990) where alveolar macrophages harvested 30 days after intratracheal instillation of silica exhibited 2 fold enhanced hydrogen peroxide secretion when challenged with zymosan. Furthermore, Vallyathan et al (1991) have reported that freshly ground silica caused significantly greater increase in hydrogen peroxide release and chemiluminescence generation than did aged silica. Excessive secretion of reactive forms of oxygen from alveolar macrophages exposed to silica may result in lung tissue destruction (Weiss & LoBuglio, 1982) as well as initiate and potentiate inflammatory responses through direct toxic effects on cells and through modification of protein, lipid, and structural components of tissues (Fantone & Ward, 1982). Therefore, silica-induced stimulation of oxidant release from pulmonary phagocytes may play a role in the development of silicosis.

Recently, PAF has emerged as a potentially crucial mediator in pulmonary inflammation, fibrosis, edema, emphysema, bronchoconstriction and bronchial asthma. Arnoux et al (1980) have reported that *in vitro* treatment with particles such as zymosan can cause PAF release from rat and rabbit alveolar macrophages. PAF release from alveolar macrophages has also been shown after *in vivo* treatment of guinea pigs with aerosolized bacterial endotoxin (Rylander & Beijer, 1987). Results from the present study are the first to indicate that *in vitro* exposure of alveolar macrophages to silica results in signigicant increase in PAF secretion (Fig. 5). Released PAF exhibits a variety of biological effects on the pulmonary tissue. PAF induces neutrophil activation resulting in an increase in lysosomal enzymes (Shaw et et al, 1981; Smith et al, 1983), secretion of reactive forms of oxygen (Van Dyke et al, 1987), arachidonic acid metabolism (Lin et al, 1982), chemotaxis (Humphrey et al, 1982), bronchoconstriction (Cuss et al, 1986; Rubin et al, 1987), thickening of the alveolar septa and pulmonary fibrosis(Camussi et al., 1983). PAF has also been shown to act directly upon endothelial cells, increasing vascular permeability (Pinckard, 1983). These results suggest that PAF involvement in the pulmonary responses to silica inhalation is possible.

In the present study, supernates from LPS-activated macrophages were used as a positive control for the thymocyte IL-1 assay. Supernates of LPS-stimulated alveolar macrophages exhibit IL-1 activity at levels 11 fold greater than the control. Silica results in a 4 fold increase in macrophage production of IL-1 activity (Fig. 6). These results on the silica stimulation are consistent with finding from other laboratories. An increase in macrophage IL-1 secretion was reported by Gery et al (1981) when peritoneal macrophages were exposed to silica for 4 or 20 h. Schmidt et al (1984) showed that IL-1 release from monocytes after *in vitro* exposure to silica was capable of regulating fibroblast proliferation. Although Driscoll et al (1989) reported that *in vitro* exposure of alveolar macrophages to silica did not activate the release of IL-1, other studies have suggested that such disagreement may be due to differences in stages of cell activation or conditions of the culture media (Rosenthal et al, 1989). Released IL-1 has been shown to stimulate fibroblast proliferation (Prostlethwaite et al, 1984; Schmidt et al, 1984), synthesis of collagens (Goldring & Krane, 1986) and glucosaminoglycans (Bronson et al, 1987). Furthermore, IL-1 may play role as an important mediator in the communication between macrophages and lymphocytes in silicosis as well. This concept implies that macrophages influence and activate lymphocytes, which then feed back to

amplify the response by stimulating the same or other recruited macrophages (Nathan et al, 1980; Unanue, 1980).

In conclusion, *in vitro* exposure of alveolar macrophages to silica activates the release of various bioactive mediators such as reactive oxygen species, PAF and IL-1 which is in line with the current understanding of the inflammation and fibrotic process after inhalation of silica.

REFERENCES

Ardlie NG, Packham MA & Mustard JF (1970) Adenosine diphosphate induced platelet aggregation in suspension of washed rabbit platelets. *Br J Haematol* **19**, 7-12

Arnoux B, Duval D & Benveniste J (1980) Release of platelet-activating factor (PAF-acether) from alveolar macrophages by the calcium ionophore A23187 and phagocytosis. *Eur J Clin Invest* **10**, 437-441

Bateman ED, Emerson RJ & Cole PJ (1982) A study of macrophage-mediated initiation of fibrosis by asbestos and silica using a diffusion chamber technique. *Br J Exp Path* **63**, 414-425

Betz SJ & Henson PM (1980) Production and release of platelet-activating factor (PAF): dissociation from degranulation and superoxide production in the human neutrophil. *J Immun* **125**, 2756-2763

Bitterman PB, Rennard SL & Hunnighake GW (1982) Human alveolar macrophage growth factor for fibroblast. *J Clin Invest* **70**, 806-822

Bronson R, Bertolami C & Siebert E (1987) Modulation of fibroblast growth and glycosaminoglycan synthesis by interleukin-1. *Coll Relat Res* **7**, 323-332

Camussi G, Pawlowski I, Tetta C, Raffirello C, Alberton M, Brenjens J & Andres G (1983) Acute inflammation induced in the rabbit by local administration of 1-O-octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine of native platelet activating factor. *Am J Pathol* **112**, 78-88

Castranova V, Bowman L & Miles PR (1979) Transmembrane potential and ionic content of rat alveolar macrophages. *J Cell Physiol* **101**, 471-480

Castranova V, Pailes WH & Li C (1990) Effects of silica exposure on alveolar macrophages: action of tetrandrine. In: *Proceedings of the International Conference on Pneumoconioses*, Shenyang, People's Republic of China.

Cuss FM, Dixon CMS & Barnes PJ (1986) Inhaled platelet activating factor in man: effects on pulmonary function and bronchial hyperresponsiveness. *Lancet* **2**, 189-191

Davis GS (1986) Pathogenesis of silicosis: current concepts and hypothese. *Lung* **164**, 139-154

Dayer JM, Goldring SR, Robinson DR & Krane SM (1979) Effects of human mononuclear cell factor on cultured rheumatoid synovial cells: interactions of prostaglandin E_2 and cyclic adenosine 3', 5'-monophosphate. *Biochem Biophys Acta* **586**, 87-105

deShazo RD (1982) Current concepts about the pathogenesis of silicosis and asbestosis. *J Allergy Clin Immunol* **70**, 41-49

Dinarello CA (1989) Interleukin-1 and its biologically related cytokines. *Adv Immunol* **44**, 153-204

Driscoll KE, Lindenschmidt RC, Higgins J & Perkins M (1988) The effects of in vivo silica and titanium dioxide (TiO_2) exposure on interleukin-1 (IL-1) and tumor necrosis factor (TNF_a) secretion by rat alveolar macrophages (AM). *The Phamacologist* **10**, p.196

Fantone JC & Ward PA (1982) Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. *Am J Pathol* **107**, 395-418

Farber JL (1982) Biology of disease membrane injury and calcium homeostasis in the pathogenesis of coagulative necrosis. *Lab Invest* **47**, 114-123

Gery I, Davies P, Derr J, Krett N & Barranger JA (1981) Relationship between production and release of lymphocyte activating factor (interleukin-1) by murine macrohpages. *Cell Immunol* **64**, 293-303

Goldring MB & Krane SM (1986) Modulation of collagen synthesis in human chondrocyte cultures by interleukin 1. *J Bone Mineral Res* **1(suppl. 1)**, p.56

Godfroid JJ & Braquet P (1986) PAF-acether specific binding sites: 1. Quantative SAR study of PAF-acether isosteres. *Trends Pharmacol Sci* **7**, 368-373.

Heppleston AG (1982) Silicotic fibrogenesis: a concept of pulmonary fibrosis. *Ann Occup Hug* **26**, 449-462

Heppleston AG & Styles JA (1967) Activity of a macrophage factor in collagen formation by silica. *Nature* **214**, 521-522

Hsueh W, Gonzalez-Crussi F & Hanneman E (1980) Pro-

staglandin synthesis in different phases of phagocytosis in lung macrophages. *Nature* **283**, 80-82

Humphrey DM, Hanahan DJ & Pinckard RN (1982) Induction of leukocytic infiltrates in rabbit skin by acetyl glyceryl ether phosphorylcholine. *Lab Invest* **47**, 227-234

Jones GS, Van Dyke K & Castranova V (1981) Transmembrane potential changes associated with superoxide release from human granulocytes. *J Cell Physiol* **106**, 75-83

Lackman LB, Page SO, Metzgar RS (1980) Purification of human interleukin-1. *J Supramol Struct* **13**, 457-466

Lin AH, Morton DR & Gorman RR (1982) Acetyl glyceryl ether phosphorylcholine stimulates leukotriene B_4 synthesis in human polymorphonuclear leukocytes. *J Clin Invest* **70**, 1058-1065

Lugano EM, Dauber JH, Elias JA, Bashey RI, Jimenez SA & Daniele RP (1984) The regulation of lung fibroblast proliferation by alveolar macrophages in experimental silicosis. *Am Rev Respir Dis* **129**, 767-771

Maridonneau I, Braquet P & Garay RP (1982) Na^+ and K^+ transport damage induced by oxygen free radicals in human red cell membranes. *J Biol Chem* **258**, 3107-3113

Martin TR, Altman LC, Albert RK & Henderson WR (1984) Leukotriene B_4 production by the human alveolar macrophages: a potential mechanism for amplifying inflammation in the lung. *Am Rev Respir Dis* **129**, 106-111

Movat HZ, Cybulsky MI, Colditz IG, Chan MKW & Dinarello CA (1987) Acute iflammation in gram negative infection: endotoxin, interleukin-1, tumor necrosis factor, and neutrophils. *Fed Proc* **46**, 97-104

Nathan CF, Murray HW & Cohn ZA (1980) The macrophage as an effector cell. *N Engl J Med* **303**, 622-626

O'Donnell MC, Henson PM & Fiedel BA (1978) Activation of human platelets by platelet activating factor (PAF) derived from sensitized rabbit basophils. *Immunol* **35**, 953-958

Pinkard RN (1983) Platelet-activating factor. *Hospit Pract* **18**, 67-76

Prostlethwaite AE, Lachman LB & Kang AH (1984) Induction of fibroblast proliferation by interleukin-1 derived from human monocytic leukemia cells. *Arthritis Rheum* **27**, 995-1001

Reynolds HY (1983) Lung inflammation: role of endogenous chemotactic factors in attracting polymorphonuclear granulocytes. *Am Rev Respir Dis* **127**, S16-S25

Rosenthal GJ, Stranahan RP III, Fort MM & Luster MI (1989) The role of alveolar macrophage activation in the response to mineral fibers. In: Mossman BT, Begin RO (ed) *Effects of Mineral Dusts on cells*, Vol H30. Berlin, Springer, 329-335

Rubin AE, Smith LJ & Patterson R (1987) The bronchoconstrictor properties of platelet activating factor in humans. *Am Rev Respir Dis* **136**, 1145-1151

Rylander R & Beijer L (1987) Inhalation of endotoxin stimulates alveolar macrophage production of platelet-activating factor. *Am Rev Respir Dis* 135, 83-86

Sauder DN, Maunessa NL & Katz SI (1984) Chemotactic cytokines: the role of leukocytic pyrogen and epidermal cell thymocyte-activating factor in neutrophil chemotaxis. *J Immunol* **132**, 828-832

Schmidt JA, Oliver CN, Lepe-Zuniga JL, Green I & Gery I (1984) Silica-stimulated monocytes release fibroblast proliferation factors identical to interleukin 1: a potential role for interleukin 1 in the pathogenesis of silicosis. *J Clin Invest* **73**, 1462-1472

Shaw JO, Pinckard RN, Ferrigni KS, McManus LM & Hanahan D (1981) Activation of human neutrophils with 1-O-hexadecyl/Octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine: the active moiety of platelet-activating factor. *J Immun* **127**, 1250-1255

Smith RJ, Bowman BJ & Iden SS (1983) Characteristics of 1-0-hexadecyl-and 1-O-octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine-stimulated granule enzyme release from human neutrophils. *Clin Immunol Immunopathol* **28**, 13-28

Unanue ER (1980) Cooperation between mononuclear phagocytes and lymphocytes in immunity. *N Engl J Med* **303**, 977-985

Vallyathan V, Kang JH, Dalal NS & Castranova V (1991) Response of alveolar macrophages to in vitro exposure to freshly fractured versus aged silica dust: the ability of prosil 28, an organosilane material, to coat silica and reduce its biological reactivity. *J Tox Env Heal* **33**, 303-315

Vallyathan V, Shi X, Dalal NS, Irv W & Castranova V (1988) Generation of free radicals from freshly fractured silica dust: potential role in acute silica-induced lung injury. *Am Rev Respir Dis* **138**,

1213-1219

Van Dyke K & Castranova V (1987) Effects of platelet activating factor on membrane potential and respiratory burst activity of human granulocytes. In: Van Dyke K & Castranova V (ed) Cellular Chemiluminescence. CRC Press, Boca Raton, Florida, Vol. II. 157-167

Van Scott MR, Miles PR & Castranova V (1984) Direct measurement of hydrogen peroxide release from rat alveolar macrophages: artifactual effect of horseradish peroxidase. *Lung Res* **6**, 103-114

Weiss SJ & LoBuglio AF (1982) Biology of disease: phagocyte-generated oxygen metabolites and cellular injury. *Lab Invest* **47**, 5-18