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CHARACTERIZATION OF ALVEOLAR MACROPHAGE EICOSANOID PRODUCTION IN A NON-HUMAN PRIMATE MODEL OF MINERAL DUST EXPOSURE

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Abstract

The relative activation of eicosanoid production which results from the exposure of the alveolar macrophage (AM) to mineral dusts is thought to be a key factor in the pathophysiology of occupational lung disease. We compared *in vitro* basal and silica-stimulated production of prostaglandin E_2 (PGE_2) and thromboxane A_2 (TXA_2) by AM from normal humans and non-human primates (Macaca nemistrina). In addition, we instilled mineral dusts directly into one lung of the non-human primate and evaluated AM eicosanoid production at two week intervals following dust instillation. Unstimulated AM from humans produce more PGE_2 and TXA_2 than do AM from M. nemistrina. However, *in vitro* exposure of AM from both species to silica dust produced a qualitatively similar increase in TXA_2 production accompanied by no change in PGE_2 production. Sequential analysis of AM eicosanoid production following a single bolus exposure to bituminous or anthracite coal dusts, titanium dioxide (TiO_2) dust or crystalline silica showed marked variability among individual non-human primates in qualitative and quantitative aspects of dust-induced eicosanoid production. However, the rank order of potency of the different dusts (silica > anthracite > bituminous) correlated with epidemiological evidence relating the type of dust mined to the incidence of pneumoconiosis. These studies suggest that the non-human primate may serve as a model for the study of both the role of eicosanoids in the etiology of dust-induced occupational lung disease and the biochemical basis for individual variability in the response of lung cells to mineral dust exposure.

Introduction

Occupational inhalation of mineral dust can lead to chronic debilitating lung disease (1,2). One of the key components of the disease process is thought to be the interaction of mineral dust with the alveolar macrophage (AM) and the subsequent release by the AM of inflammatory and fibrotic substances (3,4). Chronic inhalation of mineral dust, depending on the type and amount of dust, may then produce a situation in which ongoing inflammation, accompanied by the destruction of functional lung tissue by products of activated AM and polymorphonuclear neutrophils, stimulates the production of nonfunctional matrix by lung fibroblasts.

The AM produces several oxygenated derivatives of arachidonic acid (AA) which may play important roles in dust-induced lung pathophysiology (5,6). In previous studies in the rat, we found that prostaglandin E_2 (PGE_2), thromboxane A_2 (TXA_2) and leukotriene B_4 (LTB_4) were the major AA metabolites produced by AM (7). PGE_2 is associated with pro-inflammatory activity (8) as well as anti-fibrotic activity since it down-regulates interleukin -1 (IL-1) and tumor necrosis factor (TNF) release by AM (9-11). TXA_2 is known to stimulate platelet activity, cause bronchial and vascular smooth muscle contraction (12) and to possess chemotactic activity (13). LTB_4 is chemotactic for neutrophils and monocytes (14) and has been suggested as an additional regulatory factor in the release of TNF by AM exposed to silica dust (15).

AM eicosanoid production has been shown to be influenced by exposure to mineral dust *in vitro* and *in vivo* using both bovine and rat AM (7,16). However, the effect of mineral dusts on AM eicosanoid in the non-human primate has not been assessed despite the phylogenic proximity of this species to man and the ability to follow the progress of

dust-induced pathophysiology through longitudinal analysis of cellular and radiographic parameters. Therefore, we characterized and compared basal and silica-stimulated eicosanoid production by AM from normal humans and non-human primates. In addition, we evaluated the production of PGE_2 and TXA_2 by AM from non-human primates at several time points following a single bolus instillation of dust into the right lung. The results of these studies suggest that mineral dusts vary in their potential for activating AM eicosanoid production and that individual non-human primates vary in the reactivity of their AM to the instillation of mineral dust.

Methods

Bronchoalveolar lavage (BAL) and dust instillation -- Human AM were harvested from non-smoking, adult male volunteers by previously described methods (17,18). Briefly, the pharynx of subjects was lightly anesthetized with lidocaine and oxygen was administered by nasal prongs. Three separate aliquots of 50 ml sterile isotonic saline were passed through a flexible fiberoptic bronchoscope wedged into a subsegmental bronchus of the right middle lobe. Each aliquot was then withdrawn by gentle syringe suction. This procedure was repeated in the lingula of the left lung.

Twelve adult female pigtailed macaques (Macaca nemestrina) were used in these studies. Animals were housed in individual cages and routinely screened for bronchopulmonary infection. BAL was performed using a pediatric bronchoscope as previously described (19). In brief, 12 hour-fasted animals were anesthetized with ketamine (10-25 mg/kg, I.M.) and subjected to chest radiography, physical examination and CBC analysis. The tip of the bronchoscope was wedged into the left caudal bronchus for the lavage of lung cells. The bronchoscope was cleaned and the procedure was repeated in the right lung for either the instillation of dust or lavage of lung cells.

Characterized coal and quartz dusts used for *in vivo* exposure studies were provided by Dr. Richard Hogg, The Generic Technology For Respirable Dust. Crystalline silica (Min-U-Sil) was provided by Dr. Peter Bolsaitis, Massachusetts Institute of Technology. TiO_2 was purchased from Sigma Chem. Co. All dusts were either provided or fractionated to the same size range (2-7 μm diameter). Mineral dusts (500 mg) were suspended in 50 ml of sterile saline and instilled into the right lung of non-human primates.

At two week intervals following dust instillation, BAL was performed in order to harvest dust-exposed AM. Fifty ml of sterile phosphate-buffered saline (PBS) was instilled into the left lung and withdrawn with gentle suction. This procedure was repeated twice. The dust-exposed portion of the right lung was then lavaged in a similar manner. The BAL was performed at least twice on each animal prior to dust instillation in order to assess pre-instillation AM eicosanoid production.

BAL cell culture -- Pooled lavage fluid from each lung was filtered through sterile Nitex filters (60 µm pore size) and centrifuged (225 x g, 10 min) to sediment lung cells. Cells were washed once with PBS and recentrifuged. Lung cells were enumerated with a hemacytometer and diluted to a concentration of 5×10^5 cells/ml in tissue culture medium (M199, pH 7.35) containing 3 mg/ml albumin and 100 μg/ml L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were incubated for 1 hr (37°C in a humidified atmosphere of 5% CO_2 in air) to allow AM to attach. Non-adherent cells were removed and AM were incubated for 24 hr to allow them to return to a metabolic steady state. AM were then incubated for a final 24 hr in fresh M199 in order to evaluate either basal and silica-stimulated (100 µg/ml) eicosanoid production or the effect of *in vivo* mineral dust exposure on AM eicosanoid production. Culture medium was removed and subjected to lipid extraction in 2 x 2 vols ethyl acetate/cyclohexane (1:1). Pooled extracts from each well were dried under N_2, reconstituted in 1 ml ethanol and stored at -20°C until eicosanoid analyses were performed.

Eicosanoid radioimmunoassay (RIA) -- Appropriate volumes of extract were dried under N_2, reconstituted in BGG buffer and analyzed in duplicate for eicosanoid levels using

antibodies obtained from Advanced Magnetics, Inc. (Cambridge, MA). Antibodies were specific for the relevant eicosanoids and did not significantly cross-react with other eicosanoids (with the exception of PGE_2 antibody which cross-reacted 42% with PGE_1, a minor AM eicosanoid).

Statistical analysis -- Absolute values for AM eicosanoid production are expressed as mean (±SEM) values. Relative eicosanoid production by dust exposed AM is expressed as a percentage of values obtained from either unexposed AM from the same individual (*in vitro* exposure studies) or AM from the unexposed lung of the same individual (*in vivo* exposure studies). Comparisons of mean eicosanoid production were conducted using ANOVA in conjunction with Dunnett's procedure.

Results

Comparison of eicosanoid production by alveolar macrophages from non-human primates and humans -- We evaluated both basal and stimulated eicosanoid production by AM from humans and non-human primates in order to determine species-specific characteristics. In non-human primates we found no difference in basal eicosanoid production between AM harvested from the left and right lung. In addition, eicosanoid production by AM harvested from individual animals was similar from one lavage to another (data not shown). Basal production of both PGE_2 and TXA_2 (measured as TXB_2, the stable metabolite of TXA_2) was higher in the human than in the non-human primate (Figure 1). [These values are presented as pg/ml of culture medium since there was no difference in cellular protein content (80-100 μg) in cultures from these species.] Human AM produced 7425 (±1635) pg/ml PGE_2 and 12454 (±2030) pg/ml TXA_2 while non-human primate AM produced 2110 (±368) pg/ml PGE_2 and 422 (±92) pg/ml TXA_2. Thus, human AM produced more TXA_2 than PGE_2 while non-human primate AM produced more PGE_2 than TXA_2. The relative variability in eicosanoid production among human individuals (PGE_2 range 1610-13,320 pg/ml, coefficient of variation = 0.76; TXA_2 range 2370-22,370 pg/ml, coefficient of variation = 0.56) was similar to that among individual non-human primates (PGE_2 range 1047-4292 pg/ml, coefficient of variation = 0.58; TXA_2 range 108-995 pg/ml, coefficient of variation = 0.72).

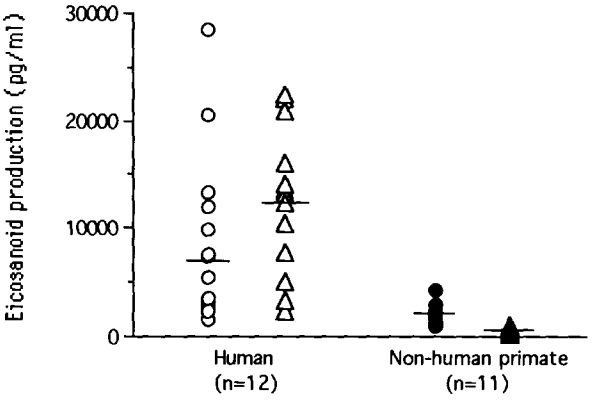

Figure 1. Basal eicosanoid production by AM from humans and non-human primates. Data points represent values for eicosanoid levels (circles are values for PGE_2, triangles are values for TXB_2) in culture medium as determined by specific radioimmunoassay. Horizontal bars represent mean values for each eicosanoid.

In vitro exposure of non-human primate AM to silica dust resulted in a significant increase in TXA_2 production from 556 (±151) pg/ml to 769 (±206) pg/ml (p=0.039, Figure 2). PGE_2 production by non-human primate AM was also increased by exposure to silica from 1257 (±176) pg/ml to 1418 (±108) pg/ml, however this increase was not statistically significant. Exposure of human AM to silica dust resulted in a modest increase in PGE_2 production from 5354 (±1706) pg/ml to 6519 (±2188) pg/ml and a substantial increase in TXA_2 production 7899 (±1918) pg/ml to 15786 (±6107) pg/ml. However, neither increase in silica-stimulated eicosanoid production by human AM was statistically significant.

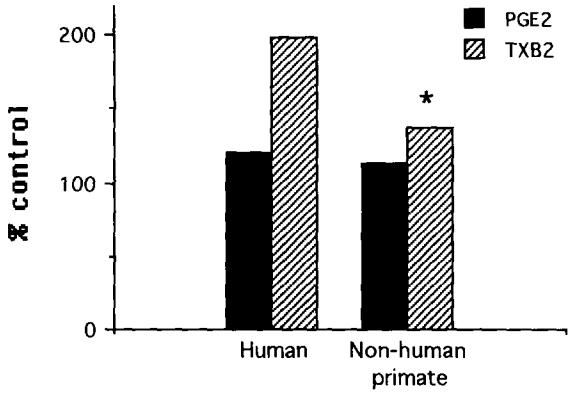

Figure 2. The effect of in vitro exposure to silica dust (100 µg/500,000 AM) on the production of eicosanoids by AM from human and non-human primates. Data are expressed as % of control (no dust exposure) values. Absolute mean values (±SEM) are reported in the text.

The effect of mineral dust instillation on eicosanoid production by AM from non-human primates -- Sequential analysis of AM eicosanoid production by AM harvested from non-human primates at 2 wk intervals following instillation of various mineral dusts suggested that considerable individual variation exists in both quantitative and qualitative aspects of the reaction to these stimuli (Figures 3-6). For example, instillation of bituminous coal into the right lung resulted in an increase in PGE_2 and TXA_2 production by AM harvested 2 weeks after dust instillation in only 1 of 3 animals (Figure 3). However, AM from another animal in this group did not react to the dust until 6 weeks after instillation. Likewise, while 1 of 3 animals instilled with anthracite coal dust demonstrated a marked biphasic response in AM eicosanoid production over the duration of the study, another animal showed only a modest reaction (Figure 4). These qualitative and quantitative differences are apparent in each group regardless of the type of dust instilled However, qualititative differences in the reaction to different dusts were observed when trends in eicosanoid production among the groups were compared (Figure 7). As expected, the greatest increase in AM eicosanoid production resulted from exposure to silica dust while, at most time points following instillation, bituminous coal dust produced only a small increase in AM eicosanoid production. In general, TiO_2 and anthracite coal dust produced an increase in eicosanoid production which was intermediate between silica and bituminous coal. There were no statistically significant differences in mean % control

values among groups, probably due to individual variability and the limited number of animals in each group. No increase in the production of eicosanoids by AM from the left lung was observed in any dust-exposed animal.

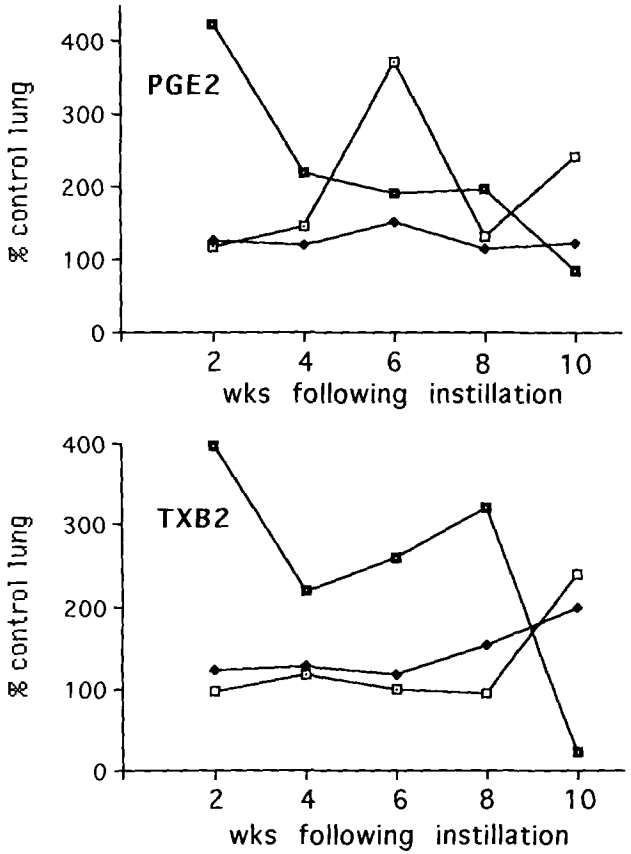

Figure 3. The effect of in vivo exposure to bituminous coal dust on the production of eicosanoids by AM from non-human primates. Different symbols represent values obtained using AM from different individual non-human primates. Values represent % change from those seen in AM from the unexposed lung of each individual.

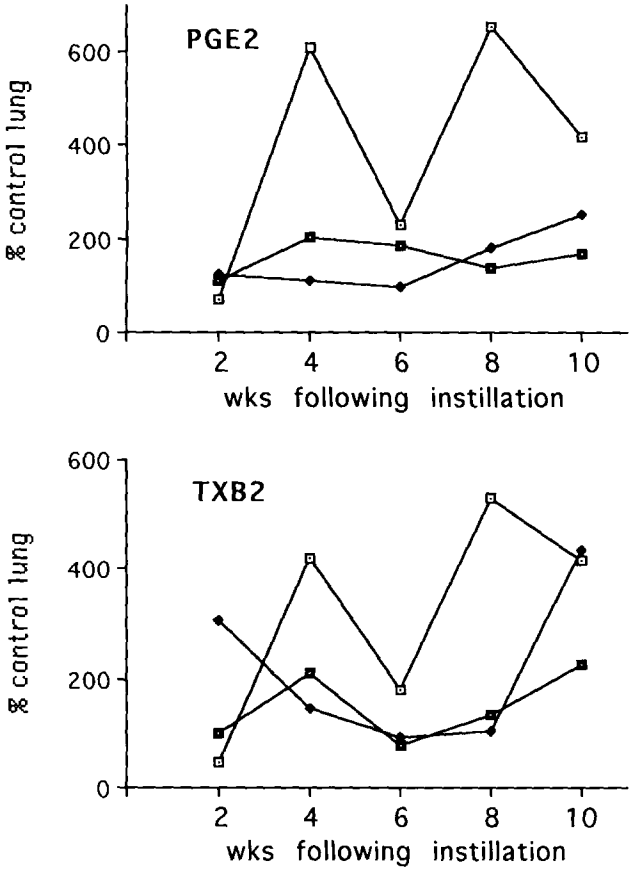

Figure 4. The effect of in vivo exposure to anthracite coal dust on the production of eicosanoids by AM from non-human primates. Different symbols represent values obtained using AM from different individual non-human primates. Values represent % change from those seen in AM from the unexposed lung of each individual.

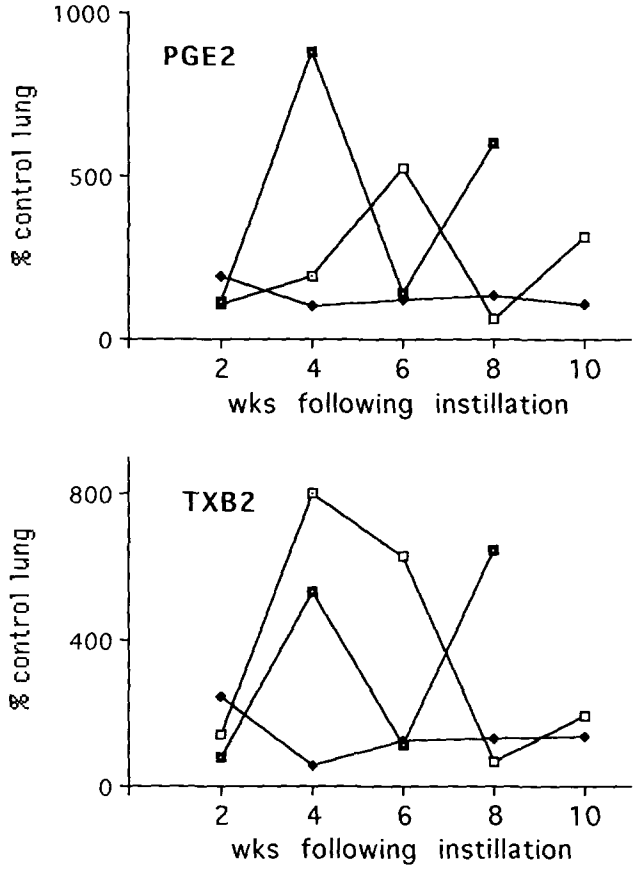

Figure 5. The effect of in vivo exposure to titanium dioxide dust on the production of eicosanoids by AM from non-human primates. Different symbols represent values obtained using AM from different individual non-human primates. Values represent % change from those seen in AM from the unexposed lung of each individual.

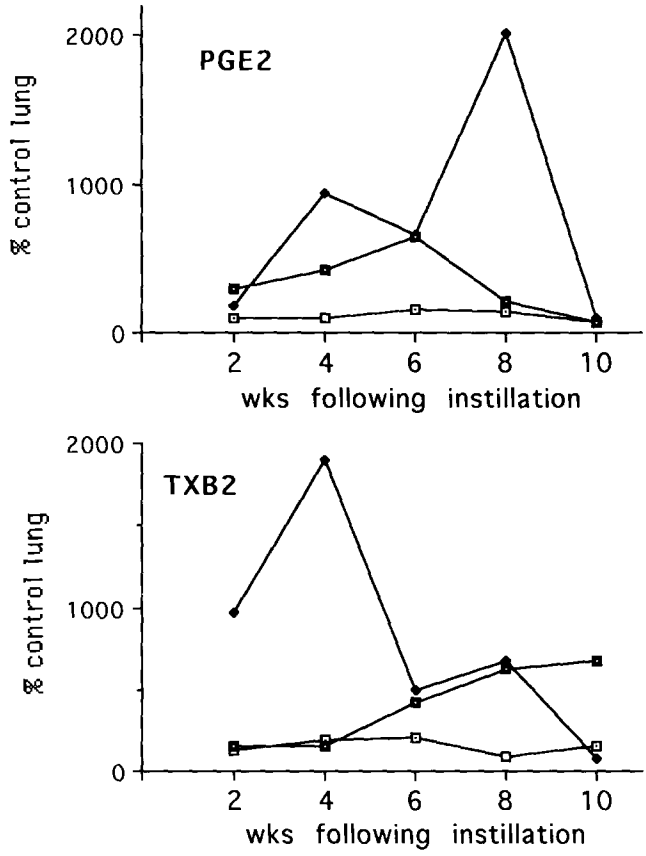

Figure 6. The effect of in vivo exposure to crystalline silica dust on the production of eicosanoids by AM from non-human primates. Different symbols represent values obtained using AM from different individual non-human primates. Values represent % change from those seen in AM from the unexposed lung of each individual.

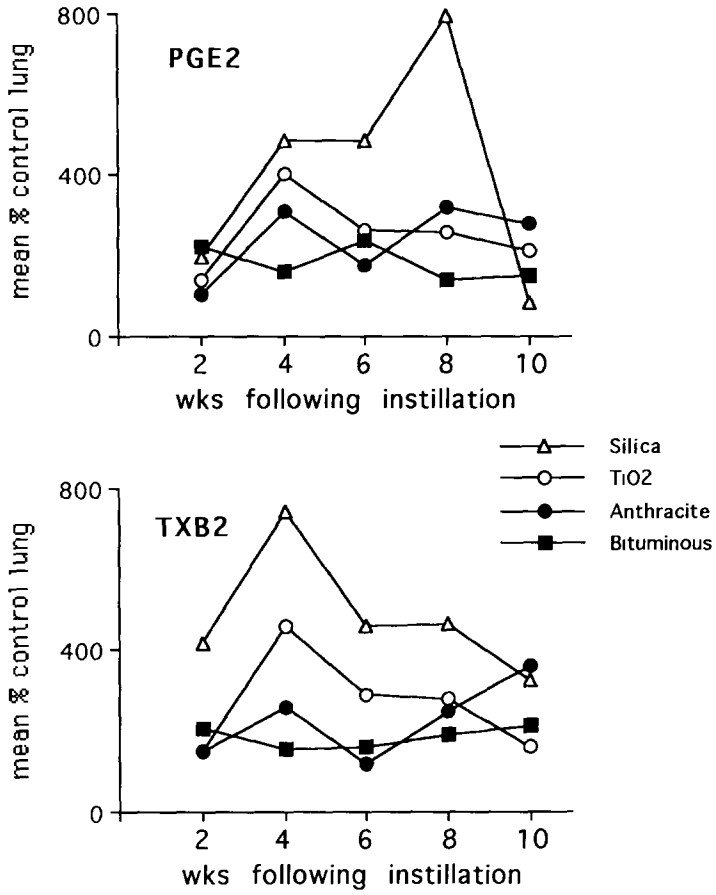

Figure 7. The effect of various mineral dusts on the production of eicosanoids by AM from non-human primates. Values at the indicated time points after dust instillation are means of values from 3 animals in each group.

Discussion

The role of macrophage eicosanoid production in a variety of inflammatory and fibrotic disease processes has been extensively evaluated. It has been suggested that the primary role of eicosanoids is expressed through regulatory functions including the mediation of vascular dynamics, immune cell recruitment, lysosomal enzyme release and cytokine production (reviewed in ref. 20). Indeed, intervention which targets exaggerated eicosanoid production (eg. aspirin) has been shown to effectively ameliorate the inflammatory process. Much of the literature related to the role of eicosanoids in inflammation has been developed using *in vitro* and animal models. These studies have been of great value in the development of therapeutic approaches in the human. However, the need for continued evaluation of these models, based on apparent species differences which may diminish their relevance to human pathophysiology and biochemistry, is well recognized. Thus, our experiments were designed to characterize AM eicosanoid release as a result of mineral dust exposure in order to provide a basis for the possible use of the non-human primate model in the evaluation of this aspect of lung pathophysiology. In particular, these studies focused on PGE_2 as a modulator of lung fibrosis through its regulatory role on macrophage cytokine release (11), and on TXA_2 production since little is known about the effect of mineral dust on the production of this major macrophage eicosanoid and since TXA_2 has been shown to be chemotactic for monocytes, the progenitor cell of the macrophage (13).

Mineral dust has been shown to activate AM eicosanoid production both *in vitro* and in inhalation and instillation models of dust toxicity (7,21-23). The mechanism of activation probably involves radical-mediated lipid peroxidation (23-25). Silica dust has been particularly well studied due to its potent biological activity and presumed key role in the occupational lung pathophysiology of coal and rock miners (1). Englen et al. (6) evaluated eicosanoid release by bovine AM exposed to 0.5-5 mg crystalline silica and found that the production of cyclooxygenase metabolites (PGE_2, TXA_2, $PGF_{2\alpha}$) predominated at lower doses of silica while lipoxygenase products (LTB_4, LTC_4) became the predominant arachidonic acid metabolites at higher doses. However, Brown et al. (26) found no PGE_2 produced by human AM exposed to 1 mg/ml silica and Bissonnette et al. (27) found no increase in PGE_2 production by rat AM exposed to 100 μg/ml silica. Finally, our own studies with rat AM showed that both amorphous fumed silica and crystalline silica can stimulate AM eicosanoid release with the former being considerably more potent than the latter (23). These disparate findings may be explained by the type and dose of silica utilized, the variable protocols employed (eg. exposure to silica immediately after harvest or following 24 hr of preincubation), the relative contribution of different subsets of AM harvested from different species (4) or the strain and age of the animals from which AM are harvested. Thus, it is apparent that the results of *in vitro* and animal model studies of mineral dust bioactivity must be interpreted carefully regarding their application to human mineral dust-induced lung disease. Studies of the effects of coal dust are much less prolific and are likely to yield even more conflicting results due to both the chemical complexity of the mineral itself and the enormous variability in mineral composition of coal from different sources.

Variability in the morphology and reactivity of AM among species and strains within species has also been reported (28,29). Moreover, the response to silica dust is apparently quite variable among strains and among individuals of the same strain. Callis et al. (30) evaluated inflammation and fibrosis in six strains of mice exposed to silica dust by intratracheal instillation and found that parameters of silica bioactivity can vary two-fold depending on the strain. In addition, Hannothiaux et al. (31) determined that lung enzymatic activity among individuals within the strain Macacus cynomolgus can vary as much as ten-fold when these animals inhale aerosolized silica dust. Thus, it is not surprising that cultured AM from both humans and monkeys showed marked individual

variability in the present study. However, despite significant differences in absolute values for PGE_2 and TXA_2 production between the two species, the relative variability within each species was not markedly different. Thus, intraspecies heterogeneity in basal eicosanoid production is similar between the human and Macaca nemestrina. It is important to note that variability in structural and functional aspects of the lung is widely thought to explain both inter- and intraspecies variability in the relative pathophysiology which may result from exposure of the lung to certain mineral dusts (32-34). Our studies suggest that the relative production of PGE_2 and TXA_2 by AM from human and non-human primates is different. That is, while the human produces more TXA_2 than PGE_2, a phenomenon also noted in the studies of Balter et al. (5), the non-human primate produces more PGE_2 than TXA_2 (similar to the rat, ref 7). The relevance of this difference to lung physiology or pathophysiology is currently unclear.

While absolute and relative aspects of basal eicosanoid production by AM from human and Macaca nemestrina showed some differences in our studies, the relative response of AM from both species to *in vitro* silica exposure was similar. TXA_2 production by AM from both species was elevated by exposure to crystalline silica while the production of PGE_2 was unchanged. The finding that human AM do not respond to silica with elevated PGE_2 production has been documented in other studies (26) and is the basis for the suggestion that the fibrotic response to silica may be due in part to the inability of the silica-exposed AM to down-regulate interleukin-1 release by increasing PGE_2 production. In contrast, PGE_2 production is elevated by *in vitro* exposure of rat AM to silica dust (23) and rats exposed to silica dust by inhalation do not develop a silicotic lesion which resembles that seen in the human (35). Thus, our data suggest that the eicosanoid response of the non-human primate AM to *in vitro* silica exposure is relatively similar to that which occurs in the human.

AM from Macaca nemestrina exhibit individual variability in response to instilled mineral dust on both a temporal and relative basis. Within each experimental group, we found individuals who showed marked elevations in eicosanoid production by AM from the exposed lung while AM from other individuals were apparently unresponsive. In addition, while AM from some animals showed a peak of eicosanoid reactivity two weeks after dust instillation (the first lavage conducted after dust administration), AM from other individuals manifested increases in eicosanoid release at later time points. In general, there was a good correlation between the patterns of production of the individual eicosanoids. This heterogeneity in AM eicosanoid response to mineral dust is similar to the variability seen in enzymatic activity by Hannothiaux et al. (31). Of interest was the observation in our studies (data not shown) that macrophage influx, as measured by total AM recovery in each lavage, correlated well with the eicosanoid response. This observation may be due partly to the chemotactic activity of TXA_2 (13) and another eicosanoid not measured in our studies, leukotriene B_4.

Examination of mean eicosanoid production among the experimental groups (Figure 7) was conducted in these studies only to begin to assess the characteristics of the model in relation to the known relative pathogenicity of different mineral dusts. While these comparisons showed no statistically significant differences among the groups, due to the apparent individual variability in response and the limited number of animals which were allocated to each experimental group, they did reveal a pattern which is similar to epidemiological associations between the type of dust mined and the incidence of pneumoconiosis. For example, McClintock et al. (36), and Jacobsen and Maclaren (37), in extensive studies of miners from British collieries, found that the silica content of coal correlated positively with the degree of progression of simple pneumoconiosis. Moreover, the rapidity with which simple pneumoconiosis progressed was proportional to the risk for the development of progressive massive fibrosis. Likewise, it has been demonstrated that, generally, the incidence of CWP is higher in anthracite coal mines than in bituminous mines (1). However, neither the role of silica nor the influence of coal rank as predominant in the pathophysiology of dust-induced lung disease is universally accepted, primarily due to the

complexity of individuals, dusts and mining environments which, in all likelihood, combine to determine the relative severity of lung malfunction and disease (38). Nonetheless, the data presented here support the concept that the type of dust to which the AM is exposed may be an important factor in eicosanoid-mediated lung inflammation. In addition, the variability among individual non-human primates supports the suggestion that host factors may be relevant to expression of the disease process resulting from inhalation of specific dust types. Thus, exposure of non-human primates to anthracite dust resulted in greater increase in eicosanoid production at most time points than did exposure to bituminous coal. In addition, silica activated AM eicosanoid production to a greater degree than either bituminous or anthracite coal dust While a statistical evaluation of the significance of differences among dusts was not possible in our studies, these results tend to support the suggestion of others than the relative ability of mineral dusts to activate AM eicosanoid production may be a key factor in the extent to which lung pathophysiology is produced.

Of additional interest in our studies was the finding that TiO_2 was not inert with regard to the activation of AM eicosanoid release. TiO_2 is thought to be innocuous and has been used in *in vitro* and animal models as an inactive, negative control dust. However, our results, as well as those of Driscoll et al. (22), suggest that TiO_2 may be biologically active under certain conditions. In particular, when dust load is high or when the AM is "primed" by prior exposure to dust or other stimulants, TiO_2 may be capable of activating AM. This activation may result in eicosanoid and cytokine release at a level comparable to that of other dusts.

The studies presented here are part of an ongoing evaluation of a variety of aspects of lung pathophysiology in the non-human primate model of mineral dust exposure. These studies will allow the sequential analysis of the effects of mineral dust on the production of mediators of inflammation and fibrosis and may provide additional valuable information related to the etiology of mineral dust-induced lung disease, the role of individual variability in the development of dust-induced lung pathophysiology, and the efficacy of potential intervention modalities designed to reduce the incidence and severity of pneumoconioses.

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References

1) Seaton, A. Coal and the lung. *Thorax* 38: 241. 1983.
2) Davis, G.S. Pathogenisis of silicosis: Current concepts and hypotheses. *Lung* 164: 139. 1986.
3) Heppleston, A.G. Silicotic fibrogenesis: A concept of pulmonary fibrosis. *Ann. Occup. Hyg.* 26: 449. 1982.
4) Fels, A.O.S., Z.A. Cohn. The alveolar macrophage. *J. Appl. Physiol.* 60: 353. 1986.
5) Balter, M.S., G.B. Toews, M. Peters-Golden. Different patterns of arachidonic acid metabolism in autologous human blood monocytes and alveolar macrophages. *J. Immunol.* 142: 602. 1989.
6) Englen, M.D., S.M. Taylor, W.W. Laegreid, H. Denny Liggitt , R.M. Silflow, R.G. Breeze, R. Wes Leid. Stimulation of arachidonic acid metabolism in silica-exposed alveolar macrophages. *Exp. Lung Res.* 15: 511. 1989.
7) Kuhn, D.C., C.F. Stanley , N. El-Ayouby, L.M. Demers. Effect of in vivo coal dust exposure on arachidonic acid metabolism in the rat alveolar macrophage. *J. Tox. Environ. Health* 29:157. 1990.

8) Downey, G.P., R.S. Gumbay , D.E. Doherty, J.F. LaBrecque, J.E. Henson , P.M. Henson , G.S. Worthen. Enhancement of pulmonary inflammation by PGE_2: evidence for a vasodilator effect. *J. Appl. Physiol.* 64: 728. 1988.
9) Scales, W.E., S.W. Chensue, I. Otterness, S.L. Kunkel . Regulation of monokine gene expression: Prostaglandin E_2 suppresses tumor necrosis factor but not interleukin-1α or β-mRNA and cell-associated bioactivity. *J. Leuk. Biol.* 45: 416. 1989.
10) Kassis, S., J.C. Lee, N. Hanna. Effects of prostaglandins and cAMP levels on monocyte IL-1 production. *Agents Actions* 27: 274. 1989.
11) Renz, H., J-H Gong, A. Schmidt, M. Nain , D. Gemsa . Release of tumor necrosis factor α from macrophages. Enhancement and suppression are dose-dependently regulated by prostaglandin E_2 and cyclic nucleotides. *J. Immunol.* 141: 2388. 1988.
13) Campbell, P.B., T.A. Tolson. Modulation of human monocyte leukotactic responsiveness by thromboxane A_2 and 12-hydroxyheptadecatrienoic acid (12-HHT). *J. Leuk. Biol.* 43: 117. 1988.
14) Martin, T.R., L.C. Altman, R.K. Albert, W.R. Henderson. Leukotriene B_4 production by human alveolar macrophage: A potential mechanism for amplifying inflammation in the lung. *Am. Rev. Resp. Dis.* 129: 106. 1984.
15) Dubois, C.M., E. Bissonnette, M. Rola-Pleszczynski. Asbestos fibers and silica particles stimulate rat alveolar macrophages to release tumor necrosis factor. Autoregulatory role of leukotriene B_4. *Am. Rev. Resp. Dis.* 139: 1257. 1989.
16) Englen, M.D., S.M. Taylor, W.W. Laegreid, R.M. Silflow, R. Wes Leid. The effects of different silicas on arachidonic acid metabolism in alveolar macrophages. *Exp. Lung Res.*16: 691. 1990.
17) Reynolds, H.Y. Bronchoalveolar lavage. *Am. Rev. Resp. Dis.* 135: 250. 1987.
18) Kuhn, D.C., J.L. Stauffer, A. Lipton, K. Leitzel, L. Gaydos, L.M. Demers. Eicosanoid and cytokine production by the alveolar macrophage from the coal miner. In: *Third Symposium On Respirable Dust In The Mineral Industry.* (R.L. Frantz, R.V. Ramani, eds.) Society For Mining, Metallurgy and Exploration, Inc. Littleton, Colo. 1992. pp.131-6.
19) Griffith, J.W., S.A. Riling. Interactions of mineral dusts and lung in a nonhuman primate model: Bituminous coal dust. In: *Third Symposium On Respirable Dust In The Mineral Industry.* (RL Frantz, RV Ramani, eds.) Society For Mining, Metallurgy and Exploration, Inc. Littleton, Colo. 1992. pp. 13-25.
20) Sibille, Y., H.Y. Reynolds. Macrophages and polymorphonuclear neutrophils in lung injury. *Am. Rev. Resp. Dis.* 141: 471. 1990.
21) Gabor, S., Z. Anca, E. Zugravu. In vitro action of quartz on alveolar macrophage lipid peroxides. *Arch. Environ. Health.* 30: 499. 1975.
22) Driscoll, K.E., R.C. Lindenschmidt , J.K. Maurer, J.M. Higgins, G. Ridder . Pulmonary response to silica or titanium dioxide: Inflammatory cells, alveolar macrophage-derived cytokines, and histopathology. *Am. J. Respir. Cell Mol. Biol.* 2: 381. 1990.
23) Kuhn, D C., L.M. Demers. Influence of mineral dust surface chemistry on eicosanoid production by the alveolar macrophage. *J. Tox. Environ. Health* 35: 39. 1992.
24) Shi, X., N.S. Dalal, V. Vallythan. ESR evidence for the hydroxyl radical formation in aqueous suspension of quartz particles and its possible significance to lipid peroxidation in silicosis. *J. Tox. Environ. Health* 25: 237. 1988.
25) Mossman, B.T., J.P. Marsh, A. Sesko, S. Hill, M.A. Shatos, J. Doherty, J. Petruska, K.B. Adler, D. Hemenway, R. Mickey, P. Vacek, E. Kagan. Inhibition of lung injury, inflammation, and interstitial pulmonary fibrosis by polyethylene glycol-conjugated catalase in a rapid inhalation model of asbestosis. *Am. Rev. Resp. Dis.* 141: 1266. 1990.

26) Brown, G.P., M. Monick, G.W. Hunninghake. Fibroblast proliferation induced by silica-exposed human alveolar macrophages. *Am. Rev. Resp. Dis.* 138: 85. 1988.
27) Bissonnette, E., B. Carre, C. Dubois, M. Rola-Pleszczynski. Inhibition of alveolar macrophage cytotoxicity by asbestos; possible role of prostaglandins. *J. Leuk. Biol.* 47: 129. 1990.
28) Murphy, M.A., H.B. Herscowitz. Heterogeneity among alveolar macrophages in humoral and cell-mediated immune responses: Separation of functional subpopulations by density gradient centrifugation on percoll. *J. Leuk. Biol.* 35: 39. 1984.
29) Haley, P.J., B.A. Muggenburg, D.N. Weissman, D.E. Bice. Comparative morphology of alveolar macrophages from six species. *Am. J. Anat.* 191: 401. 1991.
30) Callis, A.H., P.G. Sohnle, G.S. Mandel, J. Weissner, N.S. Mandel. Kinetics of inflammatory and fibrotic changes in a murine model of silicosis. *J. Lab. Clin. Invest.* 105: 547. 1985.
31) Hannothiaux, M.H., A. Scharfman, A. Wastiux, L. Cornu, E. Van Brussel, J.J. Lafitte, P. Sebastien, P. Roussel. An attempt to evaluate lung aggresion in monkey silicosis: Hydroxylases, peroxidase and antiprotease activities in serial bronchoalveolar lavages. *Eur. Respir. J.* 4: 191. 1991.
32) Rasche, B., M.T.R. Reisner, M.S. Islam, H. Thiel, I. Zimmerman, H. Baumann, W.T. Ulmer. Individual factors in the development on coal miners' pnemoconiosis. *Ann. Occup. Hyg.* 26: 713. 1982.
33) Ezenwa, A.O. Studies of environmental and host factors influencing personal differences in response to industrial silica dust exposure. *Ann. Occup. Hyg.* 26: 745. 1982.
34) Schlesinger, R.B. Comparative deposition of inhaled aerosols in experimental animals and humans: A review. *J. Tox. Environ. Health* 15: 197. 1985.
35) Heppleston, A.G., N.A. Wright, J.A. Stewart. Experimental alveolar lipo-proteinosis following the inhalation of silica. J. Path. 101: 293. 1970.
36) McLintock, J.S., S. Rae, M. Jacobsen. The attack rate of progressive massive fibrosis in British coal miners. In: *Inhaled Particles III.* (WH Walton, ed.) Unwin Bros., Surrey. 1971. pp. 933.
37) Jacobsen, M., W.M. Maclaren. Unusual pulmonary observations and exposure to coalmine dust: A case-control study. *Ann. Occup. Hyg.* 26: 753. 1982.
38) Robock, K., M.T.R. Reisner. Specific harmfulness of respirable dusts from West German coal mines. I. Results of cell tests. *Ann. Occup. Hyg.* 26: 473. 1982.

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