

Influence of Mineral Dusts on Metabolism of Arachidonic Acid by Alveolar Macrophage

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The alveolar macrophage (AM) responds to stimuli such as coal mine dust by releasing inflammatory mediators such as cytokines, growth factors, reactive oxygen species, and eicosanoids. Eicosanoids are synthesized by AM through the action of cyclooxygenase and lipoxygenase enzymes and serve to modulate the proinflammatory function of this cell as part of the lungs' host defense mechanism. Reactive oxygen species can be generated by AM as a by-product in the biosynthetic pathway of the prostaglandins. AM produces primarily prostaglandin E₂, thromboxane A₂, and leukotriene B₄ as part of the cellular response to an inflammatory stimulus. There is evidence to suggest that eicosanoid production by AM is functionally linked to both surface interaction with mine dust particles like silica and by the phagocytosis of the dust particle itself. In this report, we examined the effects of an antioxidant, vitamin E, on dust-induced synthesis of PGE₂ and TXB₂ *in vitro* and *in vivo* by AM obtained by bronchoalveolar lavage from rats. We also looked at the effects of the surface of silica particles on AM eicosanoid biosynthesis under conditions of calcination, a process that removes exposed hydroxyl groups from the surface of silica particles, thus reducing the likelihood of soluble hydroxyl radical formation. Treatment of AM with vitamin E *in vivo* and *in vitro* reduced the augmentation in eicosanoid production usually observed when AM are exposed to mine dusts. These results suggest that vitamin E may effectively reduce the inflammatory and fibrotic response produced by inhalation of mineral dust through an antioxidant mechanism. Silica that has been chemically altered by calcination was unable to activate AM eicosanoid production *in vitro* when compared to untreated, freshly fractured silica. These findings suggest that the mechanism by which dust particles can activate AM eicosanoid release may involve interaction of surface and/or soluble factors with the cell membrane. Taken together, these studies point to the involvement of AM eicosanoid production as part of the proinflammatory response of this cell to occupational inhalation of mineral dust. — *Environ Health Perspect* 102(Suppl 10):97–100 (1994)

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Introduction

Chronic and acute exposure to coal mine dust by workers can precipitate the release of inflammatory mediators from pulmonary white blood cells. The alveolar macrophage (AM) is a pivotal pulmonary defense cell in this regard that responds to the inhalation of dust particles through phagocytosis of the dust particle and the release of a number of chemical mediators that help counteract the harmful effects of inhaled foreign particles. The AM can synthesize and release a host of proinflammatory and antiinflammatory factors when activated by external stimuli. These include cytokines, growth factors, and products of arachidonic acid metabolism (1). These factors inherently send signals to other white cells through an autocrine and paracrine route to participate in the neutralization of the dust particles' effects on the lung. The AM is believed to be a key sentinel cell that can orchestrate cell to cell signaling when activated by exposure to

foreign inhalants such as coal mine dusts (Figure 1). Both blood monocytes and neutrophils are attracted into the alveolar space by eicosanoid and cytokine signals from the AM to participate and release additional inflammatory mediators in their own right. If left unchecked these inflammatory mediators and cytokines can produce the pathogenesis of lung injury. The role that the AM plays as part of the normal host defense mechanism in the pulmonary environment is an important one; however, chronic exposure to dusts and the recurring activation of these cells could lead to an unattenuated release of these potent chemical mediators of inflammation. Several of the morphologic and clinical features of the lung pathology known as coal worker's pneumoconiosis are believed to be linked to a recurrent inflammatory process.

The list of factors released by cells in the pulmonary environment under normal physiologic conditions as part of the pulmonary host defense mechanism is quite extensive. A partial list of the known factors described to date (Table 1) includes the eicosanoids, cytokines, oxygen radical species, growth factors, and unique cellular factors such as platelet activating factor. Virtually all of these factors are released from pulmonary cells when challenged by the inhalation of coal mine dusts. The cen-

tral theme of this report on dust-induced activation of the AM will focus on the eicosanoids and metabolites of arachidonic acid.

Eicosanoids

Our laboratory has been interested in the role that the eicosanoids play as chemical mediators of inflammation in the lung (2). Eicosanoids are essentially hydroxy fatty acids synthesized from the lipid substrate arachidonic acid and released from phospholipid stores found in cell membranes. The release of free arachidonic acid is the rate-limiting step in the formation of the eicosanoids and mobilization from phospholipid stores comes primarily from the influence of the enzyme phospholipase A₂. This enzyme can be activated by numerous stimuli including mine dust. The mechanism of action of glucocorticoids as antiinflammatory compounds involves inhibition of the enzyme phospholipase A₂. As shown in Figure 2, arachidonic acid is metabolized via two major pathways: a 5' lipoxygenase enzyme-mediated pathway that leads to the leukotrienes, and a cyclooxygenase enzyme pathway that produces prostaglandins, prostacyclin, and thromboxane. Once free arachidonic acid is available, the eicosanoid produced depends on the type of cell and organ system present. In the lung, the major prod-

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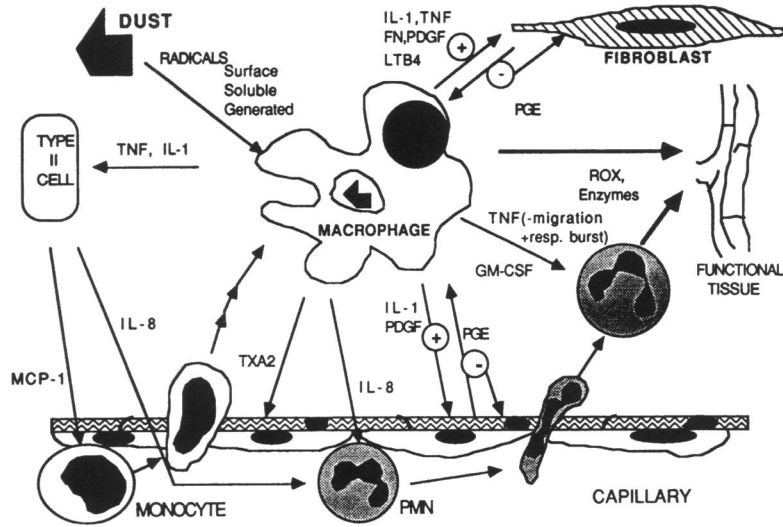


Figure 1. Multiple factors released by pulmonary alveolar macrophage upon exposure to mine dust.

ucts of the 5' lipoxygenase enzyme-mediated pathway are the leukotrienes, LTB₄, LTC₄, LTD₄, and LTE₄. Leukotrienes play a major role as chemoattractant factors in the migration and movement of neutrophils, eosinophiles, and monocytes within the lung environment. They also produce significant changes on microvascular permeability and are powerful inducers of contractility in nonvascular smooth muscle including bronchial smooth muscle.

The cyclooxygenase enzyme is part of the prostaglandin synthase enzyme complex that leads to the formation of the prostaglandins, prostacyclin, and thromboxane. The AM produces both PGE₂ and TXA₂ as major products of the cyclooxygenase-mediated pathway. PGE₂ can produce vascular smooth muscle relaxation and can elicit metabolic effects on other white

blood cells through specific receptor interaction with these cells. TXA₂ in contrast is a potent vasoconstrictor of blood vessels and causes intense aggregation of platelets and white cells in an inflammatory environment.

The biosynthetic pathway of arachidonic acid metabolism leads through a prostaglandin synthase enzyme complex that includes both cyclooxygenase and a peroxidase enzyme system that forms short-lived endoperoxide intermediates. During this reaction sequence of endoperoxide formation, reactive oxygen species can be generated. Formation of the reactive oxygen species is accomplished through a reduced pyridine nucleotide-enhanced series of reactions. Thus formation of oxygen and hydroxyl radical species can occur with activation of the AM within the scheme of

Table 1. Pulmonary cellular factors.

Eicosanoids	Proinflammatory, chemotactic, and vasoactive; PGE ₂ , PGF ₂ α, TXA ₂ , PGI ₂ , LTB ₄ , LTC ₄ , LTD ₄
Cytokines	Autocrine and paracrine factors involved in WBC and fibroblast activation and proliferation (IL-1, IL-2, IL-6, IL-8, TNF, PDGF)
Oxygen radicals	Reactive O ₂ species generated by interaction of activated pulmonary cells with dusts
Other factors	Fibronectin, PAF, lysosomal enzymes. Released as part of inflammatory process

inflammatory prostaglandin mediator release.

Mine dusts such as silica have been shown to induce a significant release in AM-derived eicosanoids (3). LTB₄, PGE₂, and TXA₂ are markedly augmented in AM in the presence of silica exposure, lending support to the suggestion that silica exposure can produce a proinflammatory mediator response from the AM.

Inhibition and Modulation of Inflammatory Mediator Release

If an uncontrolled and persistent local inflammatory event occurs in the lungs from repetitive and chronic dust exposure and if chronic AM mediator release is the predisposing cause for the development of the fibrotic changes observed in pneumoconiosis, then the use of chemical inhibitors that target several aspects of the inflammatory mediator cascade might be a rational basis for either the prevention or treatment of this pulmonary-based disorder. As shown in Figure 3, a number of chemical inhibitors that target eicosanoid production have been reported to reduce or neutralize the inflammatory mediator

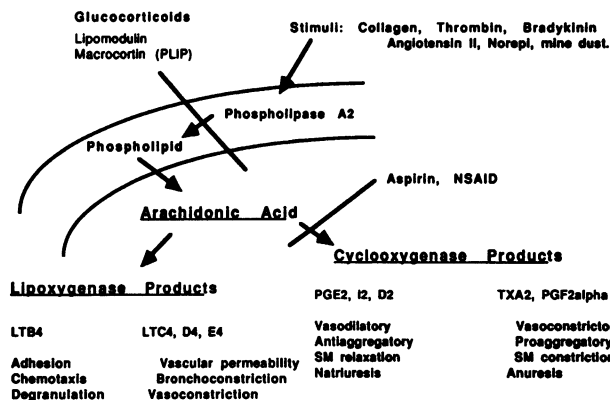


Figure 2. The metabolism of arachidonic acid to products of the cyclooxygenase and lipoxygenase enzyme-mediated pathways.

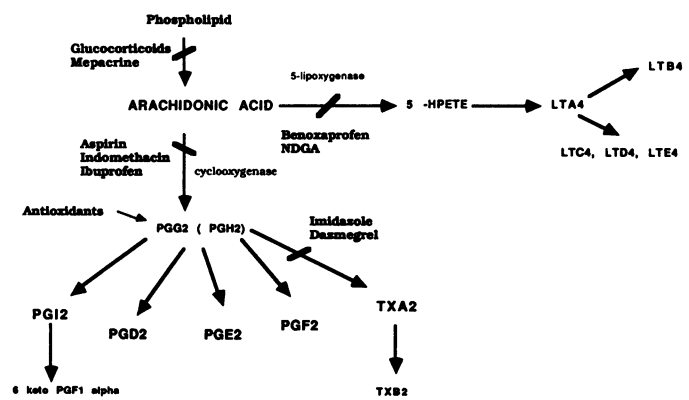


Figure 3. Inhibitors of arachidonic acid metabolism.

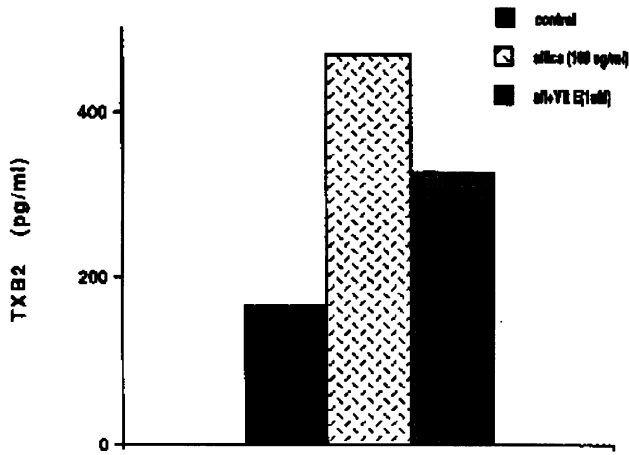


Figure 4. The effect of vitamin E on thromboxane production by AM exposed to silica dust *in vitro*.

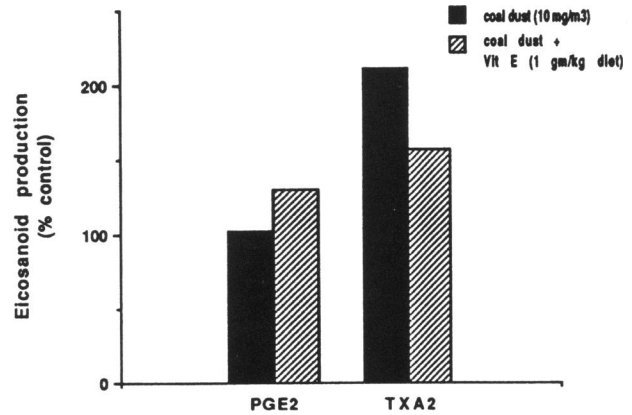


Figure 5. The effect of dietary vitamin E supplementation on thromboxane production by AM from rats exposed to coal dust by inhalation.

response to stimuli such as coal mine dust. Use of antiinflammatory compounds such as the glucocorticoids and nonsteroidal inhibitors of the cyclooxygenase enzyme such as aspirin or ibuprofen could conceivably reduce the extent of tissue damage that results from AM-induced inflammatory mediator release. Oxygen and hydroxyl radical byproducts of prostaglandin biosynthesis could also be contributory factors to the lung injury that results from chronic mine dust exposure. Thus antioxidant therapy such as vitamin E might also be an attractive means of preventive therapy. Inhibitors of the 5' lipoxygenase enzyme might also be of use in this regard; however, they have not yet enjoyed the same success as cyclooxygenase inhibitors in terms of drug development and efficacy studies.

We have performed experiments with the natural antioxidant vitamin E to determine if the augmentation in eicosanoid release from silica-exposed AM could be neutralized. Figure 4 depicts the results of an *in vitro* study in which cultured AM were incubated in the presence of silica

dust with and without the addition of vitamin E in the culture medium. As shown in Figure 4, an attenuation of the silica-induced augmentation in thromboxane release occurred in the presence of vitamin E. This suggests that oxygen radicals are linked to the release of thromboxane induced in silica-activated AM.

The idea that antioxidants such as vitamin E might prove beneficial in reducing the proinflammatory response of AM with silica exposure was further supported by several *in vivo* experiments in rats. Animals were fed diets enriched with vitamin E for several weeks prior to being exposed to coal dust in an inhalation chamber. Figure 5 depicts the results of this *in vivo* study in which rats were exposed to coal mine dust for 4 weeks in an inhalation chamber and AM recovered by bronchoalveolar lavage. Animals supplemented with vitamin E prior to dust exposure had reduced levels of thromboxane B₂ release from their AM compared to control animals. In contrast, PGE₂ release from AM was modestly increased with the vitamin E supplementation. These two studies would suggest that

antioxidant therapy like vitamin E might indeed be beneficial in reducing the level of eicosanoid release from dust-exposed AM.

Surface Property Effects of Silica on Alveolar Macrophage Chemical Mediator Release

Mineral dust interaction with AM occurs both through physical contact of the dust with the cell membrane as well as through phagocytosis of the dust particle that leads to intracellular responses from the internalization of the dust particle (4) (Figure 6). To specifically examine the physical interaction of the dust particle with the AM, we conducted a series of experiments with AM and the surface properties of silica dust and the influence of the process of calcination on dust-induced AM eicosanoid release. Calcination involves annealing the silica surface by high temperature, removing the exposed hydroxyl groups on the surface of the silica particle (Figure 7). As shown previously with red cell hemolysis activity studies, calcination effectively reduces the physical toxicity of silica at the cell membrane.

In our experiments with silica that was calcined at a temperature of 1100°C, before *in vitro* exposure to AM, a marked reduction in eicosanoid release (PGE₂ and TXB₂) from AM occurred when results were compared to silica not treated with the high temperature calcination process (Figure 8). The process of calcination

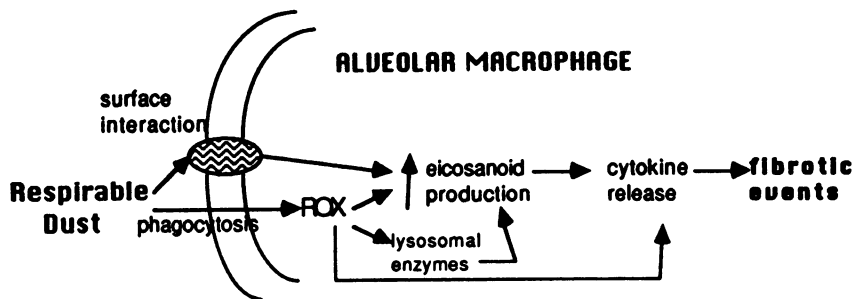


Figure 6. The phagocytic and membrane surface response of the alveolar macrophage to respirable mine dust.

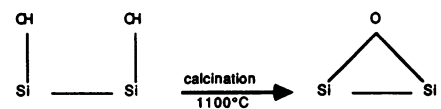


Figure 7. The effect of calcination on silica surface chemistry.

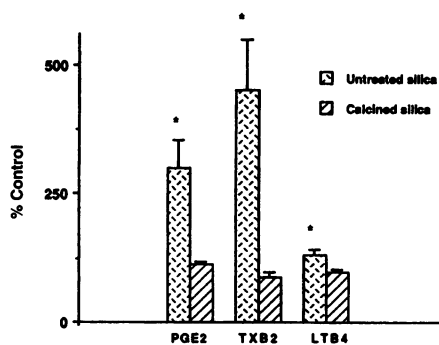


Figure 8. The effect of calcination on eicosanoid production by silica-exposed AM. * $p < 0.05$ vs control.

clearly and effectively reduced the physical effects of silica on AM eicosanoid release. This suggests that the surface elements of silica with exposed hydroxyl groups can activate the AM through direct contact with the cell membrane resulting in a turn

on of eicosanoid release. Thus the surface properties of dust appear to elicit direct activation of AM and produce metabolic events of a proinflammatory nature such as eicosanoid release.

Conclusion

When the AM comes in contact with irritating dust particles, several events occur. Physical interaction of the particles with the AM membrane can initiate a release of inflammatory mediator lipids such as the eicosanoids. This event appears to be separate and distinct from the effects associated with the phagocytosis of the dust particle by the AM and the subsequent activation of intracellular signals within the cytoplasm of the cell. The AM is pluripotent in its activation response and a myriad of factors are released as a result of both external and internal stimuli. Our previous studies *in vitro* with human cells obtained by bron-

choalveolar lavage show that both the eicosanoids and cytokines are released in substantial amounts from AM when exposed to mineral dust such as silica. It is apparent from the calcination studies that the physical properties of dust can also play an important direct role in this activation process. Studies continue in our laboratory to examine the detailed sequence of events of dust-induced AM activation and how the release of inflammatory mediators from this cell is coordinated to facilitate interaction with other cells in the pulmonary environment. The ultimate goal of this work is to identify key pharmacologic inhibitors of the inflammatory mediator cascade that target early events in the dust-induced activation of the AM. Use of these inhibitors may lead to an attenuation in the expression of proinflammatory mediators and a reduction in the lung injury that results from chronic exposure to mine dust.

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