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MECHANISMS OF PHAGOCYTIC CELL ACTIVATION BY MINERAL FIBRES

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INTRODUCTION

Chronic exposure to mineral dusts such as quartz or asbestos often causes the development of pulmonary inflammation both in humans and experimental animals. Inhalational exposure to mineral fibres results in their uptake by alveolar macrophages. Moreover, a small part of the particles is taken up also be the epithelial cells lining the pulmonary alveoli (see Doelman et al., 1990). These phagocytized, i.e. ingested mineral particles, may induce an inflammatory reaction during which chemotactic factors are released from the macrophages, and infiltration of leukocytes in the lung takes place to the site of inflammation (Lugano et al., 1982). Prolonged heavy exposure to fibrous material causes accumulation of activated phagocytic cells, i.e. neutrophils, eosinophils and basophils, in the lungs. This accumulation is accompanied with a continuous activation of macrophages. Mineral dusts are effective activators of alveolar macrophages which are the first line of defence against foreign intruders (Doelman et al., 1990). During continuous exposure to mineral fibres, macrophages play an important mediator role in the genesis of pulmonary inflammation and subsequent tissue responses. Pulmonary fibrosis is a common end result of continuous exposure to mineral fibres. Activation of macrophages results in a release of proteolytic enzymes and reactive oxygen metabolites (ROM) such as superoxide anion radical, hydrogen peroxide, the hydroxyl radical and singlet oxygen (Adamson and Bowden, 1988).

Even if the pulmonary pathology induced by long-term exposure to quartz or asbestos has been well characterized (Mossman and Marsh, 1989), little is known of the mechanisms whereby mineral fibres induce pulmonary diseases. A plethora of recent studies have provided, however, evidence that fibers stimulate phagocytic cells capable of producing ROM, and the hypothesis of oxidative stress-driven pathophysiology behind mineral fiber-induced lung diseases has gained much popularity (Mossman and Marsh, 1989). Immunological cells, alveolar macrophages, monocytes, and polymorphonuclear leukocytes (PMNL) are able to increase the production of true oxygen radicals, superoxide anion and hydroxyl radical, during the process of phagocytosis (Halliwell and Gutteridge, 1984; Seifert and Schultz, 1991).

ROM production during phagocytosis is a vital part of the normal function of phagocytic cells. The goal of ROM production, together with the release of proteases and

other secretory products, is to destroy foreign particles such as bacteria, viruses and mineral particles in phagocytic cells (Doelman et al., 1990). It is the inability of phagocytic cells to destroy mineral particles that leads to pathological persistent phagocytosis and associated production of ROM, often called frustrating phagocytosis. When excessive amounts of ROM are produced, they damage, in addition to the phagocytic cell, also surrounding cells and tissue by attacking biological macromolecules, i.e. membrane proteins and lipids, cytosolic proteins and nucleic acids (Doelman et al., 1990; Adachi et al., 1992).

Recent findings have provided evidence that nitric oxide (NO), also a radical species produced by phagocytic cells, may have a role in the activation, and in the deleterious effects of activation, of immunological cells, and in the resulting lung pathology (Moncada and Higgs, 1993). The role of NO may be especially remarkable in the controlling functions of macrophages and leukocytes. However, the mechanisms whereby NO modifies functions of phagocytic cells remain to be elucidated. The role of NO in the activation mechanisms of phagocytic cells by mineral particles will also briefly dealt with in this review.

PHAGOCYTIC CELLS AND LUNG PATHOLOGY

As described above, mineral fibres are able to induce inflammatory reaction in the lungs. Subsequent to a release of chemotactic factors, leukocytes infiltrate from the blood stream into the pulmonary tissue. Asbestos has been shown to be involved in the production of chemotactic factors (Yano, 1988). Dust-primed phagocytic cells are activated during the inflammatory process to produce ROM. A number of soluble and particulate stimuli activate alveolar macrophages and PMNL to produce ROM (von Tscharner et al., 1986; Roney and Holian, 1989; Tuomala et al., 1992; 1993a; b). Sustained production of ROM by phagocytic cells upon their stimulation by inhalable mineral fibres may be involved in a number of pathological lung conditions (Mossman et al., 1983). In a recent study, Driscoll and coworkers (Driscoll et al., 1990) demonstrated an association between increased quartz dust retention, lung injury, activation of alveolar macrophage fibronectin release and the development of fibrosis in rat lung. These and other experimental animal studies are in a good agreement with the results of a number of human studies (Mossman and March, 1989). Prolonged occupational exposure to asbestos or quartz has been specifically implicated in pulmonary fibrosis (Mossman et al, 1983; Heppleston, 1991) and in the increased risk of lung cancer in humans (Troll and Wiesner, 1985; Koskela et al, 1987). Moreover, prolonged exposure to chrysotile asbestos causes pulmonary fibrosis, bronchogenic carcinoma, and mesothelioma in humans (Mossman and Gee, 1989).

NATURE OF PARTICLE-INDUCED PHAGOCYTIC CELL ACTIVATION

Even if the mechanisms whereby asbestos, quartz or other mineral fibres, or even organic particles (Korchak et al., 1988a; Tuomala et al., 1992; 1993a; b), activate PMNL and other phagocytic cells to produce ROM have remained unresolved as yet, there is increasing evidence that this cell activation may at least partially be a receptor-mediated phenomenon. Chrysotile asbestos stimulates the production of superoxide anion in guinea pig alveolar macrophages by increasing the turnover of phosphatidic acid (PA) (Roney and Holian, 1989).

An increase in PA turnover indicates activation of inositol lipid signalling system (Berridge and Irvine, 1989). This cascade of events is associated with the elevation of the levels of free intracellular Ca^{2+} ($[Ca^{2+}]_i$), and the stimulation of protein kinase C (PKC) (Nishizuka, 1986; Berridge and Irvine, 1989; Seifert and Schultz, 1991). Tuomala and coworkers (Tuomala et al., 1992) have also shown that facilitated inositol lipid signalling precedes the production of ROM in PMNL by quartz. Thus, there are data to suggest that receptor-mediated cell signalling may be involved in particulate-induced stimulation of immunological cells to produce ROM.

ACTIVATION MECHANISMS OF PHAGOCYTIC CELLS

The mechanisms whereby mineral fibres activate phagocytic cells to produce ROM are as yet poorly known whereas those by which several soluble agonists activate the same cells are much better known. Therefore, the mechanisms of phagocytic cell activation by soluble stimuli will be discussed first, and these data are then used as a background information when reviewing the mechanisms of activation of immunological cells by mineral particles.

A chemotactic peptide, fMLP, is a bacterial chemoattractant released from bacterial membrane that causes the invasion of leukocytes to the site of the release of fMLP. Moreover, fMLP is also used as a standard stimulant to explore mechanisms of receptor-mediated activation of phagocytic cells. fMLP causes the aggregation of PMNL, depolarization of the cells, and stimulation of respiratory burst, i.e. increased O_2 consumption and increased activity of hexose monophosphate shunt (Holian and Stickle, 1985). A key-event in this process is the binding of fMLP to its cell surface receptor which through the activation of G-protein stimulates G-protein-coupled phospholipase C (PLC). This initiates a bifurcating cell signalling pathway, the production of inositol lipid-derived second messengers. Membrane phosphatidylinositol-4,5-bisphosphate is hydrolyzed by PLC to two active second messengers, inositol-1,4,5-trisphosphate (Ins-1,4,5-P_3) and diacylglycerol (DG) (Berridge and Irvine, 1984; Korchak et al., 1988a; b; Baggiolini et al., 1993). Ins-1,4,5-P_3 stimulates the release of calcium from intracellular stores (Berridge and Irvine 1984; 1989), and DG stimulates PKC (Nishizuka, 1986). PKC may phosphorylate plasma membrane NADPH oxidase which then catalyzes the reduction of oxygen to superoxide anion (Cox et al., 1985; Sadler and Badwey, 1988; Baggiolini and Wyman, 1990, Seifert and Schultz, 1991). This conclusion agrees well with the observation that in human PMNL oxidative burst correlates with the amount of PKC in the cells (Salamino et al., 1991) (see figure 1).

PMA, a tumor promoter, is a direct activator of PKC that is able to by-pass receptor stimulation (Nishizuka, 1986). PKC has a role in receptor-mediated and G-protein coupled inositol lipid signalling pathway as one of the regulatory enzymes of cellular functions and activation (Lad et al., 1992). Because of the ability of PMA to directly stimulate PKC, PMA has been widely used to explore the independent roles of PKC in various types of activation of phagocytic cells. An important feature of DG, the other key messenger in the inositol lipid signalling pathway in addition of Ins-1,4,5-P_3 (Berridge and Irvine, 1984), is that it induces a transient translocation of PKC into the membrane. This causes the activation of PKC, concomitant activation of NADPH oxidase, and subsequently increased ROM production. In contrast to DG, PMA induces a permanent translocation of PKC into the membrane, and persistent ROM production in phagocytic cells (Tuomala et al., 1993a). This difference in

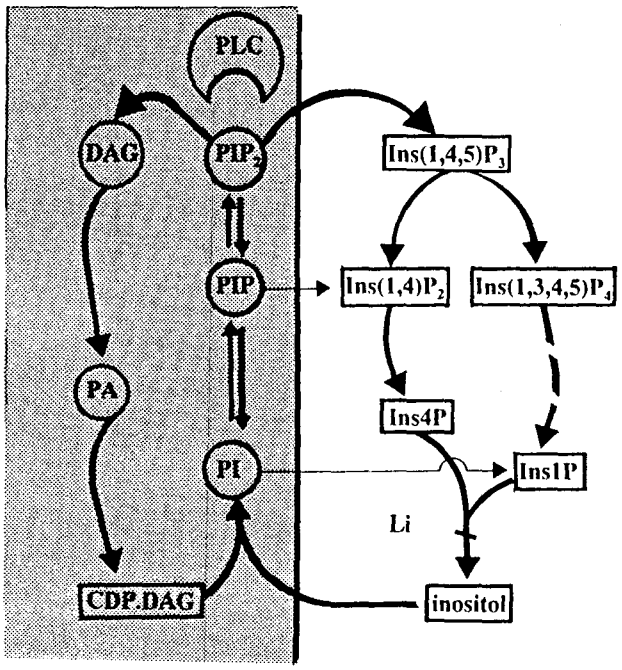

Figure 1. The hydrolysis of phosphatidylinositol 4,5-bis-phosphate (PIP_2) by phospholipase C (PLC) produces inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) and diacylglycerol (DAG). $Ins(1,4,5)P_3$ is metabolized via two alternative routes to inositol. DAG is converted to phosphatidic acid (PA) and cytidine DAG (CDP.DAG) which is utilized together with inositol forming phosphatidylinositol (PI) and ultimately PIP_2 by phosphorylation.

effects between DG and PMA may also explain some of the different responses of phagocytic cells during their receptor-mediated and direct stimulation of PKC. For example, Chabot et al. (1992) suggest that fMLP stimulates DG production through phospholipase D (PLD), whereas PMA-induced DG generation may involve several metabolic pathways including PLD activation, and activation of PA phosphohydrolase (see figure 2).

An important cellular messenger in addition to the elements of cell signalling pathways discussed above is nitric oxide (NO). NO is generated by mammalian cells through a hitherto unrecognized metabolic route by NO synthase which by using L-arginine as substrate produces citrulline and NO (Moncada and Higgs, 1993). Actions of NO are mediated through the activation of soluble guanylate cyclase and the subsequent increase in the concentration of cyclic guanosine monophosphate (cGMP) in target cells. NO is produced in large quantities also during host defence and immunologic reactions, and is generated by macrophages and PMNL (Barnes and Belvisi, 1993; Moncada and Higgs, 1993). NO is responsible e.g. for the cytotoxicity of these cells against tumor cells and bacteria. The biochemical basis for the cytotoxicity induced by NO depends on the combination of NO with iron-containing moieties in key enzymes in the respiratory chain and of the synthesis of DNA (see Moncada and Higgs, 1993). There are data to suggest that NO may also play a part both in acute and chronic inflammation, and in tissue damage. NO may not only be cytotoxic to invading particles, but to cells that produce it, and for neighboring cells (Barnes and Belvisi, 1993).

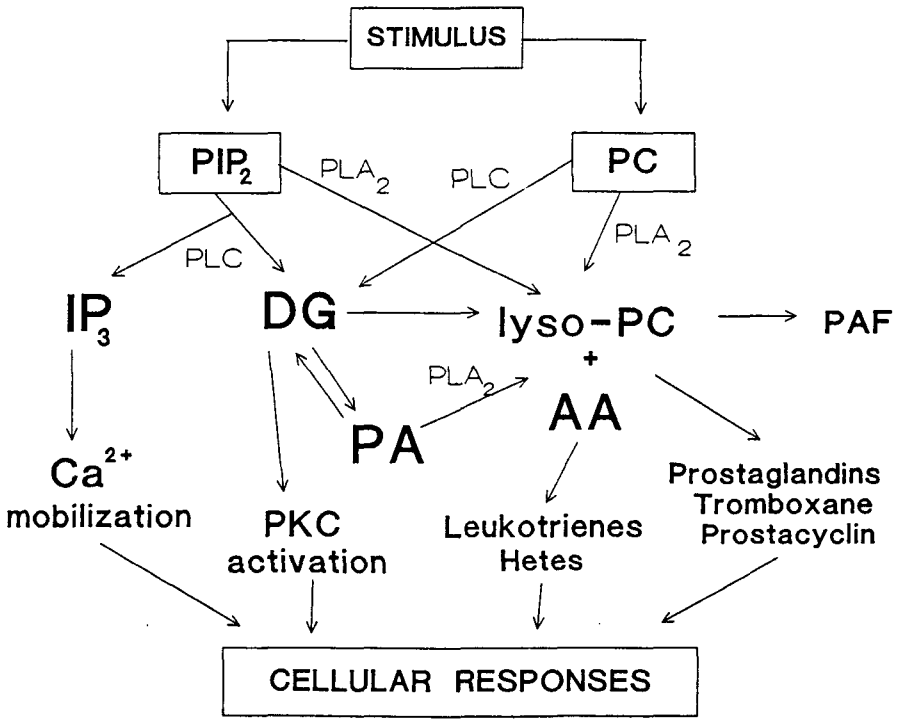

Figure 2. Summary of cellular lipid interactions. The hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) by phospholipase C (PLC) leads to the generation of two second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DG). IP_3 mobilizes calcium from intracellular stores and DG activates a phospholipid-dependent protein kinase C (PKC). The hydrolysis of phosphatidylcholine (PC) by phospholipase A_2 (PLA_2) leads to generation of lysophosphatidylcholine (lyso-PC), which is acetylated by a specific acetyltransferase to form platelet activating factor (PAF). Also PIP_2 may converted to lyso-PC. DG-kinase activates DG to form phosphatidic acid (PA) and PA-phosphohydrolase can form DG from PA. PLA_2 can activate PA to form arachidonic acid (AA), which is converted via the cyclo-oxygenase and lipo-oxygenase pathways to eicosanoids, including prostaglandins, thromboxanes, prostacyclins, leukotrienes and HETES (e.g. 5-hydroxyeicosa-tetraenoate).

Moreover, even if NO itself is a toxic radical species, it may also react with oxygen-derived radicals to generate molecules, such a peroxynitrites, that amplify NO toxicity by causing tissue damage (Moncada and Higgs, 1993). It is also likely that NO may play a role in mineral fiber-induced cell activation and subsequent cellular injury.

PROTEIN KINASE C IN PHAGOCYTIC CELL ACTIVATION

Several recent observations emphasize the role of cellular lipid metabolism and subsequent activation of PKC in agonist-induced production of ROM in phagocytic cells.

Umei et al. (1993) reported that priming of neutrophils with PMA, but not with fMLP, prior to their stimulation with the same compounds causes the translocation of PKC activity into the membrane. This event is probably a prerequisite for the enzyme activity, subsequent phosphorylation of NADPH oxidase, and ROM production. Ding and Badwey (1993a; b) have shown in guinea pig neutrophils that they contain a battery of protein kinases that are rapidly activated upon stimulation of the cells by fMLP. The activity was maximal within 30 sec with this stimulus and returned to basal level within 3 min. This is consistent with the time-course of fMLP-induced increase in inositol lipid signalling in human PMNL (Korchak et al., 1988a; b), the elevation of $[Ca^{2+}]_i$, and the increase of the production of ROM (Tuomala et al., 1992; 1993a) in these cells. In contrast, stimulation of guinea pig neutrophils with PMA resulted in a diminution of these kinase activities. However, stimulation of PKC with PMA obviously stimulates another battery of protein kinases, possible various PKC (Nishizuka, 1986) isoenzymes because this stimulation induces a strong oxidative burst in PMNL that peaks between 9 and 16 min after to the stimulation (Tuomala et al., 1993a; b).

The complexity of the signalling pathway leading to the activation of phagocytic cells is further emphasized by the observation of Kessels et al. (1993) who suggested that PKC activation is not involved in fMLP-induced activation of PLD that occurs simultaneously with oxidative burst in PMNL. The activation of PLD has been shown to result in the production of PA and to play an important role in the stimulation of PMNL respiratory burst. In fact, PA derived from phosphatidylcholine has been shown to induce respiratory burst in electropermeabilized human neutrophils by acting on a downstream step of PKC. This is most likely due to direct activation of NADPH oxidase which increases the production of superoxide anion (Mitsuyama et al., 1993). This emphasizes the complexity of PMNL stimulation because PA is further metabolized to DG by PA phosphohydrolase resulting in PKC activation (see Fig. 2). In some cases, the activation of NADPH oxidase seems to be independent of PKC activation by DG.

Activation of PKC is accompanied by the activation of NADPH oxidase, responsible for fMLP-induced rapid and transient oxidative burst. PKC inhibitor staurosporine inhibited NADPH-oxidase activity of human neutrophils activated by PMA. However, this inhibitor had no effect on either the initiation or the maximal rate of superoxide anion secretion induced by fMLP, but resulted in a more rapid termination of oxidant production (Watson et al., 1991). Thus, even if PKC plays a key-role in PMA-induced activation of NADPH oxidase and subsequent oxidative burst, this does not seem to be necessarily the case in fMLP-induced receptor-mediated activation of PMNL.

HETEROGENEITY OF PHAGOCYTIC CELL ACTIVATION

Chabot and coworkers (Chabot et al., 1992) have proposed that DG production in response to fMLP stimulation is mediated via the activation of PLD because propranolol, a specific PLD inhibitor completely blocks fMLP-induced DG production. A calcium ionophore A23187 and PMA may induce DG generation that may involve more than one pathway because propranolol was not effective in inhibiting PMA-induced DG production which may occur e.g. through activation of PA phosphohydrolase, and result in DG formation. A23187 that increases Ca^{2+} influx into the cells may act through PLC by elevating Ca^{2+}, and secondarily stimulate inositol lipid signalling. Chabot et al. (1992) concluded that PLD was

also responsible for the production of PA and DG in response to all three stimuli, fMLP, PMA, and A23187. These observations further emphasize the complex interactions between PLD and PKC in the activation of phagocytic cells.

The multifocal role of PKC in the activation of phagocytic cells is demonstrated by co-existence of at least four different protein kinases in guinea pig neutrophils (Ding and Badwey, 1993a; b). Stimulation of these kinases causes the phosphorylation of the 47-kDa subunit of the NADPH oxidase system. In contrast, stimulation of these cells with PMA or A23187 or the combination of these agonists did not activate these protein kinases or phosphorylation of the 47-kDa unit of NADPH oxidase. However, involvement of several protein kinases in PMNL activation is likely because both PMA and A23187 are able to stimulate the production of ROM in human PMNL (Tuomala et al., unpublished observation).

It is crucial to understand the basic mechanisms whereby different agonists activate phagocytic cells to produce ROM. It is through modulation and amplification of these events whereby particulate stimuli induce excessive production of radical species and resultant oxidative damage in immunological and pulmonary cells, notably lipid peroxidation of cellular membranes, protein denaturation, and chromosomal damage (Adachi et al., 1992). Key events in the activation of phagocytic cells by various agonists have been shown in figure 2. Details are given in the figure legend.

MINERAL FIBER-INDUCED ACTIVATION OF PHAGOCYTIC CELLS

The ability of asbestos and quartz to activate and increase the production of ROM in human PMNL and macrophages (Hedenborg and Klockars, 1987; 1989; Klockars et al., 1990; Nyberg and Klockars, 1990a; b; c) has been convincingly demonstrated. Perhaps the most striking new feature in fiber-induced phagocytic cell activation, and ROM production, is the possible involvement of G-protein-coupled recognition sites for mineral fibres, subsequent activation of inositol lipid signalling, $[Ca^{2+}]_i$, and the activity of PKC in this cascade of events.

Meshulam et al. (1988) showed that particulate stimuli, notably serum opsonized and non-opsonized zymosan, elicited oxidative burst in PMNL that was preceded by increased production of Ins-1,4,5-P_3 and elevation of $[Ca^{2+}]_i$. The kinetics of these events were such that they clearly proposed for the first time an association between the oxidative burst and the production of inositol lipid second messengers. In the same paper, also *Candida albicans hyphae* induced similar effects in PMNL. For details of inositol lipid signalling, see figure 1.

Roney and Holian (1989) induced excessive production of ROM in guinea pig alveolar macrophages by exposing them to chrysotile asbestos. Chrysotile dose-dependently increased the production of ROM that was preceded by increased turnover of PA, a metabolite of inositol lipid-derived DG (Berridge and Irvine, 1989). Increased PA production was followed by elevations of $[Ca^{2+}]_i$. Moreover, chrysotile-induced ROM production could be inhibited with a non-specific PKC inhibitor, staurosporine, and partially blocked with pertussis toxin that inhibits a stimulatory G-protein (G_p) coupled to PLC. Therefore, these results provide evidence that chrysotile induces ROM production in guinea pig alveolar macrophages by acting through a receptor-coupled and second messenger-mediated mechanism in which a G-protein and PKC may have at least a contributing role.

Another important piece of information suggesting a role for second messengers in

mineral fibre-induced ROM production in phagocytic cells has derived from studies of Tuomala et al. (1992) who demonstrated in PMNL that quartz increased the production of Ins-1,4,5-P_3 prior to the increased production of ROM. In another study, Tuomala et al. (1993a) showed that quartz also dose-dependently elevated $[Ca^{2+}]_i$ in stimulated PMNL. The time-course of the quartz-induced $[Ca^{2+}]_i$ elevation was such that it was clear that also the changes in $[Ca^{2+}]_i$ preceded increased ROM production. This is consistent with the assumption that quartz, in PMNL, may induce the production of ROM through a mechanism which requires both the generation of inositol lipid second messengers and subsequent elevation of $[Ca^{2+}]_i$. It was notable that the quartz-induced ROM production, both in terms of time-course and dose-effect, resembled closely those induced by fMLP and PMA. This is consistent with the view that receptor-mediated mechanisms and PKC may be involved in quartz-induced PMNL activation. In the same study, chrysotile had effects on $[Ca^{2+}]_i$ and ROM production that closely resembled the effects of quartz.

MINERAL FIBRES, CALCIUM AND CELL DAMAGE

To further explore the mechanisms whereby quartz induces ROM production in PMNL Tuomala et al. (1993b) studied the effects of repeated doses of quartz, and interactions of quartz with fMLP on $[Ca^{2+}]_i$ and ROM production. These studies revealed that a single dose of quartz dose-dependently elevated $[Ca^{2+}]_i$ and ROM production, whereas such effect was not seen after repeated doses of quartz. On the other hand, quartz administered prior to fMLP did not modify fMLP-induced $[Ca^{2+}]_i$, but attenuated fMLP-induced ROM production. Priming of PMNL with fMLP prior to quartz did not affect quartz-induced $[Ca^{2+}]_i$ changes but quartz-induced ROM production was greatly amplified. These studies provide evidence that both quartz and fMLP increase the production of ROM in PMNL by mechanisms in which $[Ca^{2+}]_i$ may have a key-role. It seems possible that both $[Ca^{2+}]_i$-dependent and -independent mechanisms may account to quartz-induced ROM production in PMNL. Moreover, in a series of as yet unpublished studies Tuomala et al. (unpublished) have shown that a novel PKC inhibitor, Ro 31-7549 (Twomey et al., 1990), almost completely blocks both quartz- and chrysotile-induced production of ROM in PMNL suggesting a role for PKC in mineral fibre-induced oxidative burst. Pretreatment of the cells with cholera toxin, but not with pertussis toxin, partially inhibited quartz- and chrysotile-induced production of ROM thereby providing evidence that a cholera but not a pertussis toxin sensitive G protein may be somehow involved in quartz- and chrysotile-induced activation of PMNL to produce ROM.

Tuomala and coworkers (Tuomala et al., unpublished) have shown that both extra- and intracellular Ca^{2+} are important for quartz- and chrysotile-induced activation of PMNL. A calcium channel blocker verapamil attenuated both quartz- and chrysotile-induced elevations of Ca^{2+} suggesting that influx of Ca^{2+} through verapamil-sensitive Ca^{2+} channels may play a role in mineral fibre-induced activation of PMNL. Moreover, the steepness of quartz- and chrysotile-induced elevation of $[Ca^{2+}]_i$ was dose-dependently decreased by verapamil indicating that verapamil inhibited the influx of extracellular Ca^{2+} into the cell. These conclusions were, in fact, confirmed by similar findings when extracellular Ca^{2+} was chelated with EGTA. These data suggest that a release of Ca^{2+} from intracellular stores is the initial event in mineral fibre-induced activation of PMNL. Thus, even if both intra- and extracellular Ca^{2+} seem to be essential for PMNL activation by mineral fibres, the release of

Ca^{2+} from intracellular stores is probably essential for the initiation of the activation, and is then amplified by a slow influx of extracellular calcium (von Tscharner et al., 1986). These findings are supported by observations of Chen et al. (1991) who showed that quartz induced a dose-dependent increase in $[Ca^{2+}]_i$ in rat alveolar macrophages. Moreover, chelation of extracellular calcium by EGTA partially inhibited quartz-induced $[Ca^{2+}]_i$ elevation, LDH release, and cellular damage. Chen and coworkers (1991) concluded that elevation of $[Ca^{2+}]_i$ is a very early event in quartz-induced macrophage damage subsequent to excessive cell stimulation by fibres. These results have been, in essence, confirmed by the findings of Rojanasakul et al. (1993) who showed that quartz produced damage in rat alveolar macrophages which was associated with increased $[Ca^{2+}]_i$ and could be partially blocked by a calcium channel blocker nifedipine or by chelation of extracellular calcium by EGTA.

NITRIC OXIDE AND MINERAL FIBERS

The role of NO in mineral fiber-induced leukocyte activation has not as yet been clarified. Some studies have been carried out with particulate stimuli, such as serum-opsonized zymosan, but not with mineral fibers (Wright et al., 1989). We have conducted some preliminary studies with quartz and chrysotile. Results from these studies indicate that both chrysotile and quartz induce time-dependent NO-production, and subsequently decrease cell viability in rat macrophage cell line RAW264.7 (Tuomala and Hirvonen, unpublished data). This phenomenon is more obvious after chrysotile- than quartz-induced activation of these cells.

CONCLUSIONS

Together these data provide evidence that the activation of phagocytic cells by mineral fibres to produce ROM may take place via mechanisms which at least partially are mediated through receptor-mediated mechanisms. Involvement of a cell membrane recognition site coupled to a cholera toxin sensitive G protein is a possibility that should be explored. Nevertheless, there is convincing evidence from studies with several different cell types that various mineral fibres, such as quartz or chrysotile, are able to increase the production of inositol lipid second messengers and concomitantly elevate $[Ca^{2+}]_i$ which seems to be a prerequisite for mineral fibre-induced activation of phagocytic cells. Both intra- and extracellular calcium seem to be important for phagocytic cell activation, intracellular calcium having a role as an initiator, and extracellular calcium as an amplifier, of cellular responses. There also seems to be an intimate relationship between intra- and extracellular calcium pools. PKC seems to play a key-role in the activation of both PMNL and macrophages to produce ROM. Regulation of PKC activity is most likely significantly affected by changes in cellular calcium homeostasis, inositol lipid signalling, and activity of number of important enzymes such as PLD and PA phosphohydrolase. Production of NO may be associated with phagocytic cell activation and may modify the responses of phagocytic cells upon stimulation by mineral fibres.

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