MEDIATORS(S) FROM HUMAN MONOCYTES/MACROPHAGES INDUCED BY QUARTZ DUST DQ12 OR COAL MINE DUST TF-1 ARE LEADING TO RELEASE OF OXYGEN RADICALS FROM HUMAN GRANULOCYTES

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INTRODUCTION

Pneumoconiosis is a chronic inflammatory and fibrotic lung disease caused by long-term inhalation of quartz dust or quartz containing dust. Inhaled dust particles with a mass median aerodynamic diameter less than 5 µm may overcome the mechanisms of lung clearance and gain access to regional lymphatic tissue, hilar lymph nodes and subpleural aggregates. Here we find the typical fibrotic nodules, which consist of quartz dust laden alveolar macrophages, lymphocytes, polymorphonuclear granulocytes and fibroblasts. Alveolar macrophages seem to play a central role in the development of fibrotic tissue changes in the lung. Several authors reported that alveolar macrophages can influence the activity of inflammatory processes by production and secretion of soluble cytokines.[4,5,6,8,11] Particular attention must be paid to the activation of polymorphonuclear granulocytes (PMN), since an increased immigration of PMN in the lung tissue, especially in the early phases of pneumoconiosis, has been reported by several investigators.[3,7,8] Human PMN are potent inflammatory cells and their importance in the development of pneumoconiosis may be due to the effects of secretory products of activated PMN as f.e. collagenases, elastases, proteolytic enzymes, and especially reactive oxygen radicals (ROS).[20] Besides, activation of prolylhydroxylase, a key enzyme in collagen synthesis, by superoxide anion has been reported.[2] Therefore, we investigated whether cultivation of human monocytes/macrophages under exposure to quartz dust DQ12 or a typical coal mine dust from the Ruhr-area could lead to the liberation of mediators, which in turn could activate PMN. Since toxicity of PMN is largely dependent on their generation of free oxygen radicals, we examined the activation of the oxidative burst of PMN by supernatants of quartz or coal mine dust exposed cultures of human mononuclear cells. Furthermore, we studied the morphological changes of PMN under influence of quartz dust induced mononuclear cell supernatants with a transmission electron microscope.

MATERIALS AND METHODS

Cell Cultures

Isolation of human monocytes and PMN from the peripheral blood of healthy donors by Ficoll-Hypaque density centrifugation as well as the maturation of monocytes to cells with characteristics of macrophages have been described in detail.[17]

Dust Samples

Quartz dust DQ12 was used as fibrogenic stimulus for cultures of human mononuclear cells. This is Dörentruper crystall quartz flour (grinding No.12) with a particle size 5 µm. Furthermore a typical coal mine dust (TF-1, fraction BAT-II) from a colliery of the Ruhr-area was tested. It is characterized by a high mineral content (95 weight%), a quartz content of 10.6 weight% and a grain size distribution from 0.5 to 2.5 µm.[18] Electrocorund (BAR 3 S, particle size < 5 µm) was used as non cytotoxic control.

Detection of Free Oxygen Radicals

Measurement of formation of reactive oxygen species, in particular of superoxide anion, from activated PMN was performed using lucigenin dependent chemiluminescence (CL).[1] For this purpose we either used a 6-channel luminometer (LB9505, Berthold, Wildbad, FRG) or a microtiterplate image luminometer (C-1966, Hamamatsu Photonics Europe, Herrsching, FRG) as reported earlier.[12,14,15] Furthermore, we studied the activation of the oxidative burst of PMN applying the cytological nitroblue tetrazolium reduction capacity test (NBT test): the percentage of detectable "formazan-cells"—associated with the uptake of nitro-blue tetrazolium and its reduction to formazan—reflects the activation of PMN by oxidative processes.[10]

Biological and Biochemical Characterization of Mediators

In order to approach the nature of the mediator(s) in the supernatants of dust treated cultures of mononuclear cells we analyzed the dose effect relationship, the thermostability, and the effect of treatment of the mediators with various enzymes.[13,14] Preliminary estimation of the molecular weight of the mediator(s) involved were performed using HPLC gelfiltration techniques (TSK 2000) as reported previously.[14] In order to evaluate the cellular origin of the mediator(s) responsible for the activation of PMN we isolated monocyte depleted and monocyte enriched cell suspension and incubated them with quartz dust DQ12 (50 µg/ml, 24h). Cellular composition of isolated cell suspensions was deter-

mined by cell surface marker analysis with an Ortho cytofluorograph (model 50 H) using FITC-labelled mouse monoclonal antibodies as described earlier.[14]

RESULTS

Supernatants of quartz dust DQ12 treated mononuclear cells are capable of stimulation of human PMN to a highly significant and long lasting chemiluminescence, which reflects the release of superoxide anion from activated PMN.[1] The mediator(s) in the supernatants responsible for this effect were called "Granulocyte Activating Mediator(s)." "GRAM". However, this activation of PMN was strictly dependent on the sort and dose of dust used for production of supernatants. Highest values of CL of PMN were obtained with supernatants from mononuclear cells exposed to 50–100 µg/ml quartz dust DQ12 for 24 hours. Even the tested coal mine dust TF-1 was able to release GRAM's from mononuclear cells,[14] but in contrast to quartz dust DQ12 peak values of CL of PMN were obtained using 200 µg/ml TF-1 for production of supernatants. Interestingly, electrocorund, known for its non-fibrogenic behaviour, was unable to release GRAM's from mononuclear cells.[14] Furthermore, we analysed the influence of the time of exposure of quartz dust DQ12 to mononuclear cells on the release of GRAM's. After an incubation period of only 4 hours low amounts of GRAM's were detected. Highest values were measured after an incubation period of 24 hours.[14] After 48, 72, and 96 hours of incubation release of GRAM's was reduced to approximately 50% of the peak value obtained after 24 hours. Therefore, supernatants harvested from cultures of mononuclear cells exposed to 50 µg/ml quartz dust DQ12 for 24 hours were used as "standard-GRAM" for further characterisation of GRAM. Exposure of PMN to "standard-GRAM" in the NBT-test led to a threefold increase in the formation of formazan-cells.[12,14] This result again underlines the activation of the oxidative burst of PMN by GRAM's. We also analyzed the morphological changes of PMN exposed to GRAM with a transmission electron microscope. We found, that in contrast to control cells PMN which were exposed to GRAM for 15 min. present marked signs of chemotactic activity as can be seen by changes of the cell shape and development of a leading lamella. After an incubation period of 45 min. PMN seem to enlarge to some extent. After 60 min. of incubation we found a reduction of chemotactic activity. Additionally, we detected a loss of intracytoplasmatic granules, indicating the release of lysosomal products.[12,14] In further studies we investigated some biological and biochemical properties of GRAM. The chemiluminescence inducing activity of standard-GRAM was detectable up to a dilution 1:16.[14] Activity of GRAM was progressively diminished by heat treatment and was abolished after boiling of supernatants.[14] After treatment of GRAM with hydrolytic enzymes and subsequent testing of the remaining CL induction on PMN, it was demonstrated that GRAM is relatively stable towards ribonuclease, neuraminidase and trypsin, whilst chymotrypsin and protease significantly reduced the activity.[13,14] Preliminary estimations of the molecular weight of GRAM using HPLC gelfiltration techniques indicated that the chemiluminescence inducing activity of GRAM is probably caused by two substances with a m.w. of about 10 kDa and 20 kDa resp.[14] In further studies we investigated the cellular origin of GRAM. Therefore, we incubated monocyte enriched and monocyte depleted cell suspensions with 50 µg/ml quartz dust DQ12 for 24 hours. Supernatants were harvested and tested on their ability to induce chemiluminescence of PMN. Results demonstrated that monocyte depleted cell cultures (95% lymphocytes, 5% monocytes/macrophages) were unable to release sufficient amounts of GRAM. Cultures of mononuclear cells consisting of 17% or 50% of monocytes/macrophages were strong inducers of release of GRAM.[14] Data suggest that monocytes/macrophages and not lymphocytes are the main producers of GRAM.

DISCUSSION AND CONCLUSION

Results presented demonstrate that human monocytes/macrophages in culture release a soluble mediator(s) following incubation with quartz dust DQ12 or coal mine dust TF-1. The mediator(s) stimulates human PMN to the release of reactive oxygen species, especially of superoxide anion. Therefore, we named this mediator(s) "Granulocyte Activating Mediator(s)," "GRAM". Some important characteristics of GRAM are summarized in Table I: Human monocytes/macrophages are able to release GRAM's if stimulated with low amounts of fibrogenic dust particles (quartz dust DQ12 or coal mine dust TF-1 were tested). The relative thermoresistance of GRAM and the sensitivity of GRAM against treatment with protease or chymotrypsin suggest a protein nature of GRAM. Preliminary determination of the molecular weight of GRAM indicates that two molecules (or two parts of one molecule) with a m.w. of approximately 10 kDa and 20 kDa resp. are responsible for the observed chemiluminescence. Besides the enhancement of the oxidative burst of PMN by GRAM we observed the induction of stong chemotactic changes as well as the release of lysosomal products from GRAM treated PMN in a time dependent manner by ultrastructural analysis. Taken together our results present an *in vitro* example of possible non-direct mechanisms of quartz- and coal mine dust pathogenicity. Monocytes/macrophages, when treated with low amounts of quartz dust DQ12 or coal mine dust TF-1 release a soluble cytokine like mediator(s), which then in turn activates human PMN to production of reactive oxygen species. Oxygen species could either directly be toxic and thus lead to alveolar damage[9,16] or could enhance activity of prolylhydroxylase, a key enzyme in collagene synthesis.[2] Furthermore, the chemotactic effects of GRAM on PMN may explain the immigration of PMN in the early phases of silicosis.

In previous studies we reported that human monocytes /macro-phages under influence of quartz- or coal mine dust release a "Fibroblast Proliferation Factor," "FPF".[17,19] The question whether FPF and GRAM are identical has not yet been investigated. Therefore, further research concerning biological and biochemical properties of the mediators described is needed. Our investigations as done so far point to new non-direct, cytokine mediated mechanisms of pneumoconiosis. Measurement of the release of such

Table I

Important Characteristics of Quartz and Coal Mine Dust Induced Granulocyte Activating Mediator(s)

GRANULOCYTE ACTIVATING MEDIATOR (GRAM)	
Source:	Human monocytes/macrophages
Inducing Agent:	Quartz dust DQ12, Coal mine dust TF-1
Molecular Weight:	- one (part of a) molecule with m.w. about 20 kda - another (part of a) molecule with m.w. just <10 kda (preliminary estimation, reduced by data from HPLC gel-filtration)
Stability:	56°C, 60 min - 37% loss of activity 80°C, 60 min - 58% loss of activity 100°C, 60 min - 81% loss of activity
Sort of molecule:	Protein nature
Target cells:	human polymorphonuclear granuloctes (PMN)
Effects:	Induction of chemiluminescence of PMN Formation of Formazan cells morphological changes of PMN
Results:	- Metabolic activation with release of oxygen radicals (superoxide anion) from PMN - chemotactic activation of PMN

mediators may be helpful to estimate the noxious effects of respirable particles.

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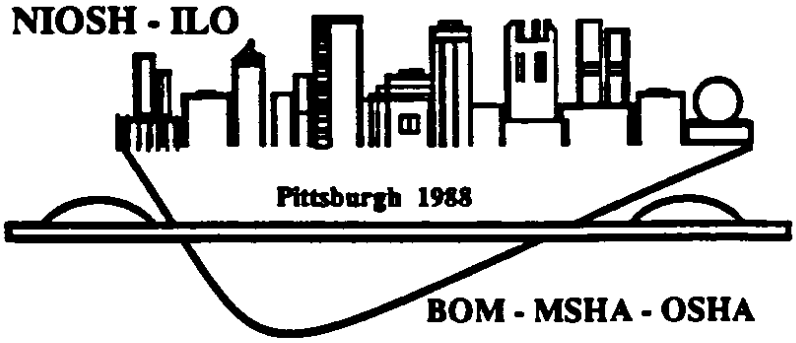
II



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