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CYTOTOXIC EFFECTS OF DUST PARTICLES ON ALVEOLAR MACROPHAGES

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ABSTRACT

Alveolar macrophages (AM) play a central role in mobilizing body defence system against inhaled pathogens. AM gained by lung lavage of healthy calves were used. They were separated from erythrocytes, lymphocytes and dead cells using discontinuous Percoll gradients. Human AM from healthy adults were purified in the same way. AM were incubated with airborne dust particles of various concentrations for different periods of time. The degree of damage was assessed by means of a rosetting assay with IgG opsonized erythrocytes demonstrating the efficiency of the Fc receptor. The dust particles revealed a strong cytotoxic effect in the range of 50 to 200 μg/ml. AM were also incubated with heavy metal compounds which themselves were dust constituents. The cytotoxic effect was linear in a dose dependent manner between 0 and 20 μmol/l as shown for Cd^{2+} in Fig. 4. In contrast Hg^{2+} inhibited the rosetting in the range of 1 to 5 μmol/l.

KEYWORDS

alveolar macrophages; airborne dust particles; cadmium; mercury; Fc receptor; rosetting assay; discontinuous Percoll gradient.

INTRODUCTION

As part of the mononuclear phagocytic system alveolar macrophages (AM) play a central role in body defence system against inhaled particles. Damage to these cells caused by toxicants from the environment can lead to an increased susceptibility to pulmonary infections. AM are responsible for different effector functions of the immune system in the lung such as phagocytosis of opsonized cells (Bowden, 1984). These processes are mediated by cell surface receptors like the Fc receptor (Comber *et al.*, 1989). Therefore, we investigated the effect of airborne dusts on the function of the Fc receptor. In order to evaluate the influence of airborne dust constituents AM were also incubated with heavy metal compounds, and the efficiency of the Fc receptor was assessed by means of a rosetting assay.

MATERIALS AND METHODS

Bronchioalveolar cells, gained by lung lavage of freshly slaughtered, approximately 6 months old, conventionally raised, healthy calves, were used. The AM were separated from erythrocytes, lymphocytes,

epithelial and dead cells using discontinuous Percoll gradients (Pharmacia Chemicals, Uppsala, Sweden, specific gravity 1.09, 3500 rpm, 20 min, 4 °C). A purity of 98% was achieved. The cell moiety was divided into two parts. One part was sowed in petri dishes and cultivated overnight in Dulbecco's Minimum Essential Medium (DMEM, Biochrom, Berlin) containing 100 units/ml penicillin, 100 mg/l streptomycin (Biochrom) and 50 mM HEPES buffer (Serva, Heidelberg). The other part was cultivated in suspension in the same medium. The cells were prevented from adhering by rolling them in 15 ml pp tubes (Greiner, Nürtingen). Airborne dusts were sampled within the AFR-atmospheric control program in Hamburg (Arbeitsgemeinschaft zur Förderung der Radionuklidtechnik 1983) on cellulose acetate filters. Element analysis was carried out during the AFR air dust program by W. Dannecker and co-workers. For incubation the dusts were suspended in DMEM by ultrasonication as described elsewhere (Gulyas and Gercken, 1988). In parallel experiments AM in suspension and adherent AM were incubated with dust suspensions in the range of 50, 100, 150 and 200 μg/ml for 12 and 24 h. The same experiments were repeated with HgO and CdO suspensions as well as with $HgCl_2$ and $CdCl_2$ (p.A. Merck, Darmstadt) solutions in different concentrations. All samples were gently shaken during the incubation periods. The culture medium was removed and the cells were washed three times. The degree of damage was assessed by means of a rosetting assay with IgG opsonized erythrocytes (Behringwerke, Marburg). The rosettes (Fig. 1) and the non-rosetting cells (Fig. 2) were counted.

All described experiments were also carried out with human AM from healthy adults, purified and treated in the same way.

Fig. 1. Rosetted AM

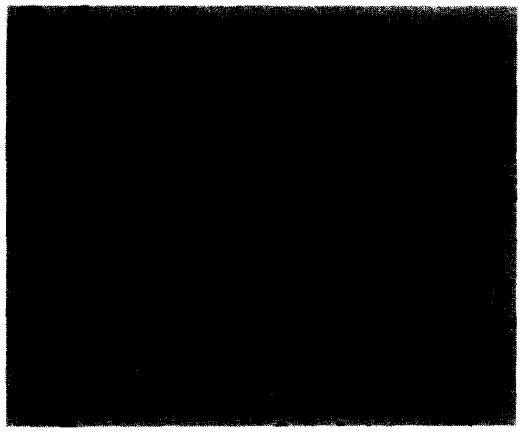

Fig. 2. Non-rosetted AM

RESULTS

In all series of tests the dust particles revealed a strong cytotoxic effect on AM in the range of 50 to 200 μg/ml. In addition AM which had ingested extraordinarily many dust particles showed a total inhibition of rosetting. There was a remarkable difference between adherent AM and AM in suspension. The latter showed in all experiments under the same conditions a higher degree of damage than the adherent cells (Fig. 3). Even the rosetting AM were not comparable to the control rosettes. As positive cells we counted AM with more than 3 bound erythrocytes to each macrophage. The inhibition of rosetting increased to the same extent as the degree of mortality. In order to achieve a higher rate of differentiation AM were incubated with certain heavy metal compounds that are constituents of the dust. The investigated heavy metal compounds showed a cytotoxic effect between 0 and 20 μmol/l at 12 h incubation. This effect was linear in a dose dependent manner as shown for Cd^{2+} in Fig. 4. The rosetting AM were comparable to the

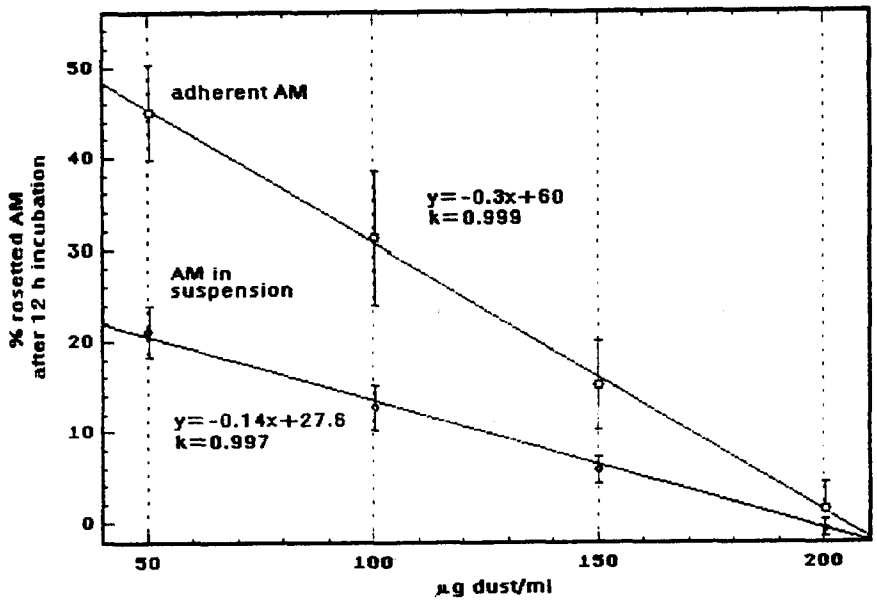

Fig. 3. Difference between adherent rosetting AM and rosetting AM in suspension after dust incubation.

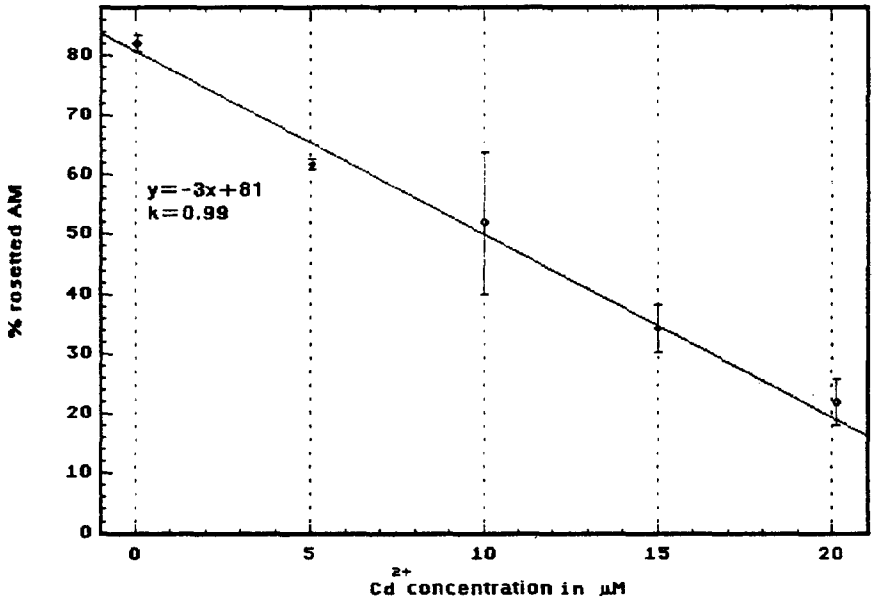

Fig. 4. Rosetting AM (%) after 12 h of incubation with Cd^{2+} in different concentrations.

control rosettes. The inhibition of rosetting was caused by dead cells as determined by Trypan blue exclusion. In contrast Hg^{2+} inhibited the rosetting in the range of 1 to 5 μmol/l. This effect could be recognized after 1 h of incubation. The ratio of vital cells/dead cells corresponded to the controls.

CONCLUSION

Fc receptor alteration measured by inhibition of antibody-mediated rosette formation is a sensitive parameter by which adverse effects of environmental toxicants can be measured (Hadley *et al.*, 1977). The observed cytotoxic effects of dusts correspond to the intracellular toxicity of the most heavy metal cations in a dose dependent manner. We suggest for Hg^{2+} a different biological mechanism because Hg^{2+} inhibits the rosetting in very low non-toxic concentrations.

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