Abstract—Using a rat model we set out to determine whether exposure of bronchoalveolar-derived leukocytes to pathogenic mineral dusts in vitro caused them to undergo an oxidative burst and release potentially harmful oxidants. Three different populations, obtained by bronchoalveolar lavage, were chosen: control cells, cells obtained following instillation of heat-killed *Corynebacterium parvum* into the lung, and cells obtained following instillation of quartz. None of the populations showed any evidence of superoxide anion or hydrogen peroxide production when treated in vitro with the pathogenic dusts quartz and chrysotile asbestos, or the inert particulate titanium dioxide. Zymosan caused modest release of superoxide anion with all three populations, indicating that a respiratory burst was being provoked, while PMA, a soluble inducer of leukocyte oxidative burst, caused large-scale production of both oxidants. Preopsonization of mineral dust in rat serum did not render them capable of provoking an oxidative burst from lung-derived leukocytes.

INTRODUCTION

Occupational exposure to certain mineral dusts is associated with the development of pulmonary inflammation (1–4) and fibrosis (5). Experimental deposition of pathogenic mineral dusts, principally quartz and asbestos, in the lungs of laboratory animals has confirmed their ability to cause alveolitis (6, 7) and fibrosis if exposure is persistent (8, 9). This dust-induced parenchymal fibrosis has resulted in the inclusion of the pneumoconioses among the interstitial lung diseases which are typified by persistent alveolar inflammation and, at the end stage, fibrous tissue accumulation in the interstitium (10). The cell biology of the interstitial lung diseases has been extensively studied, and the leukocytic inflammatory cells present in the lung have been implicated in the alveolar dam-

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age which leads to fibrosis (11, 12). Oxidants are one class of leukocyte product which are considered to be likely arbiters of tissue damage during persistent inflammation (13, 14). However, release of oxidants by lung-derived leukocytes on exposure to mineral dusts has not been systematically studied.

The present study was undertaken to assess, using a rat model, the oxidant produced by three different populations of bronchoalveolar cells in response to mineral dust. The three populations constitute bronchoalveolar cells relevant to the study of mineral dust alveolitis. These populations were: control, 16 h after intratracheal instillation of heat-killed *Corynebacterium parvum*, five days after intratracheal instillation of quartz dust. The release of superoxide anion and hydrogen peroxide, produced during the respiratory burst of the leukocytes, was measured following in vitro exposure to the non-inflammation-generating dust titanium dioxide (15) and two dusts known to cause alveolitis, quartz and chrysotile asbestos. Standard particulate and soluble triggers of the respiratory burst were included for comparison.

**MATERIALS AND METHODS**

*MATERIALS.* Cytochrome c, dextrose, phorbol myristate acetate (PMA), horseradish peroxidase, hydrogen peroxide, superoxide dismutase, and zymosan were obtained from the Sigma Chemical Company, Poole, Dorset. *Corynebacterium parvum* (heat-killed) was obtained from Wellcome Reagents, London. Dulbecco’s phosphate-buffered saline (PBS) and F10 medium were obtained from Gibco Limited, Paisley, Renfrewshire. Nembutal was purchased from Ceva Limited, Watford, Hertfordshire. Plastics were obtained from Sterilin, Feltham, Middlesex.

*ANIMALS.* PVG rats, inbred under SPF conditions at the Institute of Occupational Medicine Animal Unit, were used throughout.

*Dusts.* The following dusts were used: titanium dioxide (rutile; Tioxide Limited, Stockton on Tees, Cleveland, England), DQ12 quartz, chrysotile asbestos (UICC standard sample A).

*Intratracheal Instillation.* Rats were anesthetized with ether and the trachea exposed by blunt dissection. A blunt-ended needle was introduced into the trachea down to the carina, through a small incision. A volume of 0.2 ml containing 1.4 mg of heat-killed *Corynebacterium parvum* or 1 mg of DQ12 quartz was injected, and the skin closed with metal clips. To obtain an acute neutrophil-rich exudate, the rats injected with *C. parvum* were killed 16 h later; to obtain the dusted macrophage/neutrophil population, the DQ12 rats were killed five days later.

*Bronchoalveolar Lavage.* Rats were killed by intraperitoneal injection of Nembutal. The lungs were dissected free from the thoracic cavity and lavaged with 3 × 10 ml sequential volumes of saline at 37°C. The lavaged cells were kept in plastic tubes on ice during counting and preparation of cytocentrifuge smears.

*Superoxide Anion Assay.* Superoxide anion was measured according to the method of Johnston (16). The reaction buffer (phosphate-buffered saline, PBS), containing 1 mg/ml cytochrome c and 2 mg/ml dextrose) was prepared and 1.5 ml were added to 30-mm petri dishes. Bronchoalveolar lavage cells were prepared at 5 × 10⁶/ml in PBS and 50 μl of cells (0.25 × 10⁶) were added to the reaction buffer in dishes. The particulates quartz, chrysotile asbestos, titanium dioxide, and zymosan were prepared in PBS at 2 mg/ml and diluted so that the addition of 50 μl to each petri