Release of Superoxide Anion and Hydrogen Peroxide by Macrophages in Response to Asbestos

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Introduction

The multiple roles of the macrophage in the inflammatory response are well documented (Nathan et al. 1980). In particular the importance of reactive oxygen intermediates (ROI) during inflammatory defence against microbes has been recognized (Klebanoff 1980). However, it has also been suggested that inappropriate ROI release by phagocytes during non microbially induced inflammation may, under some circumstances, prolong and exacerbate tissue damage and inflammation (Fantone and Ward 1982). The possibility that the deposition of pathogenic dusts in tissue could result in just such a build up of potentially toxic ROI has been suggested (Gee and Walker-Smith 1984). Using mouse peritoneal macrophages elicited with asbestos we have found support for this contention in the raised oxidative status of asbestos activated macrophages as measured by chemiluminescence (Donaldson and Cullen 1984). In the present paper we report on the levels of superoxide anion and hydrogen peroxide released by asbestos-activated macrophages. We also describe the effect of hydrogen peroxide and superoxide anion on the functional activity of lymphocytes as an indicator cell population.

Materials and Methods

Animals and Treatment

The animals used were inbred male C57BL6 mice, 8 - 12 weeks old at the time of use. Mice were injected intraperitoneally with 0.5 ml sterile Dulbecco A (Dul A); up to 2.5 mg chrysotile asbestos (Union Internationale Contre Cancer. Sample A); up to 2.5 mg DUL A, or 0.4 mg Corynebacterium parvum (Wellcome).
Cells

Peritoneal exudate cells were harvested 5 days after injection with 5 ml of DUL A + 10 U/ml Heparin (Leo Laboratories) and kept on ice. The cells were washed and resuspended in F10 medium + 10% Heat Inactivated Foetal Calf Serum (Gibco) plus antibiotics at 10⁶ cells/ml. One ml of this suspension was added to 30 mm plastic petri dishes (Sterilin) and allowed to adhere for one hour at 37° C in 5% CO₂. Just prior to assay the cells were washed three times with DUL A to remove non-adherent cells.

ROI Measurement

Superoxide anion was assayed according to the method of Johnston (1981) with phorbol myristate acetate (PMA: Sigma) present at 1 µg/ml as trigger; superoxide dismutase controls were always included. Hydrogen peroxide was measured according to the method of Pick and Keisari (1980) with PMA (1 µg/ml) present as trigger.

Cellular Toxicity of ROI

To test for toxic effects of ROI at levels similar to those produced by the asbestos elicited macrophages, exogenous hydrogen peroxide, and an enzymic superoxide generating system were used. These were added to splenocytes proliferating in response to mitogen and the effect of ROI on thymidine uptake was measured by liquid scintillometry. The splenocyte proliferation assay used was that described by Donaldson et al. (1984) except that phytohaemagglutinin (PHA: Sigma) at 10 and 50 µg/ml, was used as mitogen. Hydrogen peroxide (Sigma) was added to cells to concentration of 500, 100, 20, and 5 µM. To generate superoxide anion a xanthine (Sigma: 50 µg/ml) / xanthine oxidase (Sigma: 10 mU/ml) mixture was used; by cytochrome C reduction this system generated 11.6 n.moles of superoxide anion/0.5 h.

Statistics

Data were examined by analysis of variance and paired t' tests.