Asbestos Inhalation and the Induction of Splenic Lymphocytic Proliferation in the Rat

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There is evidence that the alveolar macrophage plays a critical role in the pathogenesis of asbestosis, since this phagocytic cell is an important component of evolving lesions in this disease. We have recently demonstrated that alveolar macrophages obtained from asbestos-exposed rats can evoke a stabilized physical interaction during in vitro culture with autologous T cell-enriched splenic lymphocytes. This interaction was shown to have two components: an effect analogous to that produced by subjecting macrophages to the peroxidant action of sodium metaperiodate (NaI04) and an effect which was similar to antigen-directed clustering of lymphocytes around macrophages. As an extension of the previous study we now report that alveolar macrophages obtained from asbestos-exposed rats can initiate a vigorous in vitro splenic lymphocyte proliferative response.

Materials and Methods

Adult male BD-IX strain rats were exposed by intermittent inhalation to UICC crocidolite asbestos (1350 fibers/ml: 10.3 mg/m8) for six months (dusted rats). A matched group of BD-IX rats received no asbestos exposure (control rats). Alveolar macrophages and nylon-column purified, T cell-enriched splenic lymphocytes were obtained from both control and dusted rats.

A series of lymphoproliferative assays were performed, in which alveolar macrophages were co-cultured with autologous or syngeneic splenic lymphocytes from dusted and control rats in a "checkerboard" fashion. The experimental design is illustrated in Figure 1.

Results

Macrophages from dusted rats induced significant lymphoproliferation after 72 hours (P < 0.001) or 96 hours (P < 0.001) in either autologous lymphocytes or lymphocytes from syngeneic control rats, when compared with co-cultures containing macrophages from control rats and lymphocytes from either control or dusted rats. The proliferative response induced by macrophages from dusted rats in lymphocytes from control animals peaked after 72 hours (15440 ± 368 dpm) and was abolished by pre-treatment of the macrophages with L-cysteine (3168 ± 279 dpm). A similar effect could be shown by pre-treating alveolar macrophages from control rats with NaI04.

The blastogenic response induced by macrophages from dusted rats in autologous lymphocytes peaked at 96 hours (11838 ± 2515 dpm). This response was not abrogated by L-cysteine (11805 ± 1657 dpm).

Discussion

The present study has demonstrated that protracted asbestos inhalation in the rat produced two distinct effects on the alveolar macrophages from dusted rats: (1) a periodate-like effect akin to surface membrane peroxidation (which was observed in co-cultures of macrophages from dusted rats and lymphocytes from control animals); (2) an effect produced by a putative asbestos-related, macrophage-associated neeanitigen (which was only observed in co-cultures of macrophages from dusted rats with autologous lymphocytes). The periodate-like effect could be abolished by pre-treatment of the macrophages with the reducing agent, L-cysteine. The antigen-like effect resulting from asbestos inhalation could not, however, be abrogated by L-cysteine. The induction of a proliferative response in splenic lymphocytes by autologous alveolar macrophages from dusted rats occurs subsequent to the sustained autologous lymphocyte clustering around these macrophages, which was noted in an earlier study. These observations parallel those noted during the activation of lymphoproliferation by soluble protein antigens.

The in vitro sensitization of rat splenic lymphocytes to inhaled asbestos could result from the ready access of inhaled fibers to the pulmonary microcirculation, as illustrated by the presence of asbestos fibers within endothelial cells in the lung shortly after inhalation. Our findings have important implications regarding the pathogenesis of asbestosis. The induction of cellular immunity in the lung to inhaled asbestos might initiate a complex series of reactions involving the interplay of lymphokines, lysosomal hydrolytic enzymes and com-

Figure 1. Experimental design of the study. 3H-TdR = triitated thymidine.

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plement activation, with pulmonary fibrosis reflecting the end-stage of these events (Fig 2).

REFERENCES


DISCUSSION

Dr. Fox: Did you see neutrophils that could be releasing oxygen radicals and oxidizing the surface of the macrophages?

Dr. Kagan: No. Less than 3% of the total lavage cell population were neutrophils.

The Use of Pulmonary Washings as a Probe to Detect Lung Injury


Many in vitro tests for mutagenicity and cytotoxicity have been developed to provide a means for rapidly evaluating the potential health risks of environmental pollutants. For airborne materials, such as fibers, tests cannot take into account the effect of route of entry on the risk to tissue nor can in vivo tests measure the integrated response of the whole animal to an inhaled pollutant. Therefore, to bridge the gap between short-term in vitro tests and long-term animal studies, we have developed a short-term, in vitro screening test in which analysis of lung washings is used to detect an acute inflammatory response in the lungs of exposed animals.1

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