REVERSIBILITY OF FIBROTIC LESIONS IN RATS INHALING SIZE-SEPARATED CHRYSOTILE ASBESTOS FIBRES FOR 2 WEEKS

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INTRODUCTION
Inhaled asbestos fibres cause lung disease in both humans and animals. Fibre inhalation results in the development of cellular injury, inflammation and tissue remodelling. Epithelial proliferation and excessive collagen deposition are major features associated with this process. Alterations in lung tissues at alveolar duct bifurcations have been noted following exposures as short as 1 h to aerosolized asbestos fibres (Brody et al., 1981; Warheit et al., 1984; Chang et al., 1988). One consequence of this pattern of fibre deposition is a highly localized and active region of epithelial injury and repair with the proliferation of alveolar type II cells and interstitial cells. These changes progress during the first 48 h following exposure and continue to be evident 1 month after fibre exposure has ended.

The long-term consequences of limited exposure to asbestos fibres are not as well defined. Although a number of studies have examined exposure to asbestos fibres and the sequelae over a long period of recovery for lung tumor incidence, there have been no studies which have quantitatively assessed tissue changes occurring during a long postexposure period or following short-term exposures to chrysotile asbestos fibres. The purpose of this study was to examine the consequences of short-term exposure to aerosolized chrysotile asbestos fibres over a 1 year period of recovery in the lungs of rats. This study specifically addresses the issue of whether the initial changes seen immediately following asbestos exposure continue to persist or to progressively worsen with longer periods of recovery.

METHODS
Groups of male Crl:CDBR rats (7–8 weeks old, Charles River Breeding Laboratories, Kingston, New York) were exposed by inhalation 6 h day⁻¹, 5 days week⁻¹ for 2 weeks to mean fibre concentrations of 458 and 782 fcc⁻¹ and assessed at several postexposure time periods. After completion of exposures, the lungs of chrysotile-exposed animals and aged-matched sham controls were assessed for asbestos-induced histopathological effects immediately after exposure, as well as 5 days, 1, 3, 6 and 12 months postexposure.

Animals were anesthetised with an intraperitoneal injection of sodium pentobar-
Fig. 1. Bronchiole–alveolar duct junction (BADJ) in longitudinal profile. The terminal bronchiole and a pair of alveolar ducts with an intervening tissue ridge or first alveolar duct bifurcation is present. BADJs and the central acini from the lungs of rats 1 week after a 2 week exposure to asbestos fibres. Thickening of alveolar septal tips and walls is illustrated (arrows) and was most extensive through 4 weeks, but showed a reduction at 13 weeks which continued through 1 year postexposure. Scale bar is 250 μm.

Materials and Methods

Bital and the lungs fixed by intratracheal instillation of glutaraldehyde at a hydrostatic pressure of 20 cm of fixative. Within 48 h, tissue slices from the lungs were embedded in paraffin. Sections 5–6 μm thick were cut with a rotary microtome and stained with hematoxylin and eosin, or sirius red for histologic examination. Tissue sections were examined microscopically and bronchiole-alveolar duct junctions (BADJs) were identified. Selection of BADJs was based on the criterion of a terminal bronchiole opening directly onto the longitudinal profile of an alveolar duct (Fig. 1). In many instances, the first alveolar duct bifurcation formed by the tissue ridge separating two alveolar ducts arising from the same terminal bronchiole was also present.

Tissue sampling and morphometric analysis

Morphometric analysis was carried out only on the high dose (782 fec−1) animals. Each BADJ selected was examined for the presence of delicate, lacy tissues forming the alveoli of the duct wall along with regions showing marked thickening. Alveolar wall thickening was defined as an increase in the cellularity and volume of tissues forming the alveolar septal tip, the opening forming the entrance from individual alveoli to the alveolar duct lumen (Fig. 1).

To determine the extent of changes occurring within the central acinus following exposure to fibres, the distance from the most distal portion of the alveolar duct
showing thickening of the alveolar septum to the BADJ was measured for each isolation. The relative proportion of alveolar septal tips with thickening of 50% or greater compared to control tissue was also determined for each isolation.

Alveolar septal tissues within the lungs of animals exposed to fibres that had increased in thickness by 50% or more, compared with lung tissues of control animals were selected for further analysis. Each region was photographed at a magnification of 1500×. Using the program, Stereology Toolbox, each tissue field was analyzed using a test lattice system consisting of 21 lines, each 2.25 cm in length, placed at random over each image. The number of times the ends of each line fell over tissue, capillary, or alveolar macrophage was recorded. The number of intercepts made by each test line with the air–tissue interface was also counted. Measurements were compiled for all alveolar septal tips analyzed from a minimum of 4 BADJs per animal. Tissue and alveolar macrophage volumes were normalized to the alveolar surface area using the formula,

\[ V = \frac{S}{\delta} \]

where \( V \) is the volume fraction derived from the number of points falling on structures of interest and \( S \) is the surface fraction derived from the number of test line intercepts with the air-to-tissue surface within alveolar tissue areas analyzed.

The presence of collagen within lung tissues was analyzed in tissue sections stained with sirius red in picric acid solution. Sirius red is an azo dye which is highly selective for the staining of fibrous collagen. The distribution of collagen fibrils and bundles throughout parenchymal tissues and thickened alveolar septal tips was determined at each time period examined following exposure to fibres.

RESULTS

Repeated exposure to asbestos fibres over a 10-day period resulted in significant changes occurring in alveolar ducts immediately beyond the terminal bronchiole. These changes under the exposure regimen used in our study were not confined to only first alveolar duct bifurcations, but extended along the length of the alveolar duct. The BADJ could be easily identified along with numerous prominent thickenings of alveolar septal tips and bifurcations along the length of the alveolar duct forming the pulmonary acinus (Fig. 1). The most prominent changes within the alveolar duct noted as increased cellularity and septal wall thickening, was evident during first month after fibre exposure had ended. With greater postexposure time, the prominence of septal tips with increases in the cellularity and thickness of alveolar walls, decreased significantly (Fig. 2).

The volume of alveolar tissues formed by septal tips and walls and normalized to alveolar surface area is shown in Fig. 3 for control and asbestos-exposed animals. Measurements taken from the lungs of rats exposed to asbestos fibres were confined to only those septal tips and alveolar walls found to be increased in cellularity and thickness. Thickening of alveolar septal tissues was almost doubled compared with that seen in control animals immediately following as well as 5 days and 1 month following completion of the exposure. By 3 months postexposure, a significant reduction was noted in the volume of affected alveolar tissues in the lungs of exposed animals. Although alveolar septal tips and walls were on average thicker than those seen in control animals, the differences between control and treated animals were not statistically significant. Alveolar tissue volume, even in
the most affected regions of the lungs of animals exposed to fibres did not change significantly from 3 months to 1 year after the end of exposure. Over this same period of time, tissue volumes in control animals did not change significantly.

The volume of macrophages normalised to alveolar surface area was significantly elevated through the first 3 months following exposure to aerosolised fibres (Fig. 4). A five-fold elevation above control values was noted 1 month after the end of exposure. Following 3 months recovery, macrophage volume was significantly decreased. No significant differences were noted in the volume of macrophages 26 or 52 weeks after fibre exposure compared with control values.

DISCUSSION

This study illustrates that the inhalation of asbestos fibres over a short period of time leads to significant changes within the alveolar tissues of the lung. These changes consist of increased cellularity and thickening of the alveolar walls and septal tips which form the alveolar duct immediately beyond the BADJ. These alterations were most prominent immediately following exposure, but by 1 month postexposure, changes had reached their maximum extent and had begun to resolve. This pattern had been noted in earlier studies with prominent changes persisting 1 month after the end of exposure. From our studies we found that further recovery time from exposure to the fibres resulted in a significant reduction
Fig. 3. Alveolar tissue volume (mean ± SEM) following exposure to asbestos fibres. All measurements are normalised to the alveolar surface area for comparison. An asterisk denotes a statistically significant difference ($P < 0.05$) compared with control.

and some resolution of the injury and remodelling of the tissues associated with fibre inhalation. These consisted of a reduction in the cellularity of the alveolar tissues, particularly those areas that were most affected initially by exposure to asbestos fibres. Although there remained a slight thickening of septal tissues, these were not statistically significant from that noted in control animals. As early as 3 months following exposure, tissue changes had shown resolution with no further progression 1 year after the end of fibre exposure. The distance into the alveolar duct in which tissue changes were seen did not change (data not presented). The proportion of the alveolar duct wall involved in tissue changes was significantly reduced as early as one month after the end of exposure. These findings suggest that with the inhalation of asbestos fibres, there is extensive involvement of the lung parenchyma that is not confined to the first alveolar duct bifurcation. These changes are typical of what has been reported in the past. This study demonstrates that with repeated exposures to high levels of respirable fibres over the short-term does not lead to a progression and a worsening of injury over the long-term period of recovery as originally speculated. There is significant resolution of the initial tissue changes that were noted up to 1 month following the end of fibre exposure. This resolution appears to occur as early as 3 months following exposure and does not show progression to a more severe state 1 year after the end of fibre exposure.
Fig. 4. Alveolar macrophage volume (mean ± SEM) following exposure to asbestos fibres. All measurements are normalised to the alveolar surface area. An asterisk denotes a statistically significant difference (P < 0.05) compared with control.

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