Environmental Health Perspectives
Vol. 51, pp. 85-89, 1983

Interactions of Chrysotile Asbestos with Erythrocyte Membranes

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Chrysotile asbestos causes lysis of red blood cells. It has been proposed that the mechanism of hemolysis is mediated through interactions between asbestos and cell membrane glycoproteins. Our studies support this concept and the following results are reported. Electron microscopy shows that asbestos fibers distort red blood cells and bind to cell membranes which may become wrapped around the fibers. This reaction is prevented by pretreatment of the cells with neuraminidase. The distribution of lectins which bind to membrane glycoproteins is altered by treating the cells with asbestos. Cell distortion and membrane deformation consequent to asbestos treatment correlate with a clear increase in the ratio of intracellular Na^+:K^+ ions.

Introduction

Chrysotile is the most toxic asbestiform mineral (*1*). This asbestos type causes fibrotic and neoplastic lung disease consequent to inhalation by animals (*2*) and man (*3*). The mechanisms through which chrysotile asbestos exerts its toxic effects have been speculated upon (*1,4,5*). One reasonable hypothesis is that positively charged magnesium ions on the surface of the asbestos fibers bind to negatively charged sialoglycoproteins on cell membranes (*1*). This has been tested in erythrocyte systems where hemolysis is measured (*6*) and in cultured cells studied biochemically (*7*). We have addressed this hypothesis further by studying the distribution of erythrocyte membrane glycoproteins before and after treatment with chrysotile asbestos. The significance of changes in cell surface glycoprotein distribution is assessed by correlative studies of intracellular Na^+ to K^+ ratios.

Materials and Methods

Blood was drawn (through cardiac puncture) from white rats into a syringe and diluted 1:10 in a solution of 0.15 *M* NaCl, 5*mM* glucose and 5 *mM* KCl. Red cells (RBC) were cleaned by repeated centrifugation and finally suspended in 0.05 *M* Tris with 0.15 *M* NaCl. RBCs were fixed in diluted Karnovsky's fixative and critical point dried for scanning electron microscopy.

Gold spheres measuring approximately 20 nm in diameter were prepared by refluxing 4% $HAuCl_4$ (0.25 mL) and 1% Na citrate (2 mL) in H_2O (100 mL) (*8*). This creates a colloidal suspension of gold spheres which was dialyzed overnight against 0.005 *M* Tris HCl (pH 7.3).

Gold conjugated wheat germ agglutinin (Au-WGA) was prepared according to Horisberger and Rosset (*9*). WGA was crosslinked to BSA with glutaraldehyde and then incubated with the dialyzed colloidal gold for 15 min. The lectin from *Limulus polyphemus* was incubated directly with the gold colloid in 0.005 *M* Tris (pH 7.3) to provide a cell marker (Au-Lim). The gold-conjugated lectins finally were suspended in a solution of 0.15 *M* NaCl, 50 *mM* Tris HCl containing 0.4 mg/mL of polyethylene glycol (Carbowax). The Limulus lectin was held in 0.01 *M* $CaCl_2$ prior to use.

To remove sialic acid from RBC surfaces 10^6 RBCs were incubated in a solution of 0.15 *M* NaCl, 0.05 *M* Tris HCl, 0.01 *M* $CaCl_2$ (pH 7.0) containing 1 IU of neuraminidase [from *V. cholerae* at 37°C for 1 hr (*10*)].

The intracellular ratios of Na^+:K^+ ions were determined in freeze-dried RBCs. Cells exposed to asbestos for 5 and 15 min and unexposed time-matched controls were spread thinly on a carbon disk with a wooden stick and plunged into liquid nitrogen. The disks then were transferred to a freeze-drying apparatus for 5 hr at − 50°C. Individual cells were analyzed by X-ray energy spectrometry (XES) for Na^+

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and K^+ content in a JEOL 100 CX STEM. The ratio of the number of Na^+-derived X-rays to K^+ X-rays was calculated through computer programming.

Suspensions of 0.2 g aliquots of chrysotile asbestos in 200 mL distilled water were sonicated (45 min) and centrifuged (200*g*) to separate large fibers. Of the fibers remaining in suspension, 95% had diameters less than 0.2 μm and lengths less than 6 μm as determined by SEM. RBCs were treated with these fibers at a concentration of 0.1 mg/mL in 0.15 *M* NaCl and 0.05 *M* Tris HCl (pH 7.3).

Results and Discussion

Red Blood Cells and Asbestos

Light microscopy and scanning electron microscopy showed that over 80% of untreated RBCs exhibited a normal biconcave morphology after 4 hr in Tris-buffered saline (Fig. 1*a*). Addition of chrysotile asbestos led to distortion and deformation of 75-80% of the cells within 15 min of treatment (Fig. 1*b*). Asbestos fibers were intimately associated with RBC membranes as has been shown previously by Craighead et al. (*11*). It appeared as though portions of the membranes were drawn around asbestos fibers (see Figs. 3 and 4).

Pretreatment of RBCs with neuraminidase (NA) protected over 75% of the cells from the deforming effects of asbestos (Fig. 2). Even though the NA-treated cells were surrounded by asbestos, the normal morphology was maintained. These findings correlate with earlier studies where it was shown that neuraminidase decreased the hemolytic potential of chrysotile asbestos (*1,6*). We have illustrated that early asbestos-induced membrane distortions are prevented by neuraminidase. Such RBC deformation could be integral to the hemolytic process. The protection imparted by neuraminidase strongly suggests a role for negatively charged sialic acid in mediating membrane binding to positively charged chrysotile asbestos (*1,6*). The significance of membrane and cell deformation is considered below.

RBCs and Wheat Germ Agglutinin (WGA)

SEM clearly illustrated that gold-conjugated wheat germ agglutinin (Au-WGA) was evenly distributed across the surface of normal RBCs (Fig. 3*a*). This reaction was blocked by prior treatment of RBCs with wheat germ agglutinin. Pretreatment of the cells with chrysotile asbestos severely altered the normal Au-WGA distribution pattern on distorted cells (Fig. 3*b*). The number of Au-WGA labeled sites per unit area of asbestos-reacted RBC surface was reduced to less than 30% of the control level.

WGA is known to bind to *n*-acetylglucosamine and *n*-acetylneuraminic (sialic) acid on cell membranes (*12*). Our findings suggest that these membrane carbohydrates are altered in quantity and/or distribution by the deforming effects of chrysotile asbestos. Treating RBCs with crystalline silica served as an important control inasmuch as silica-induced hemolysis reportedly is not mediated through

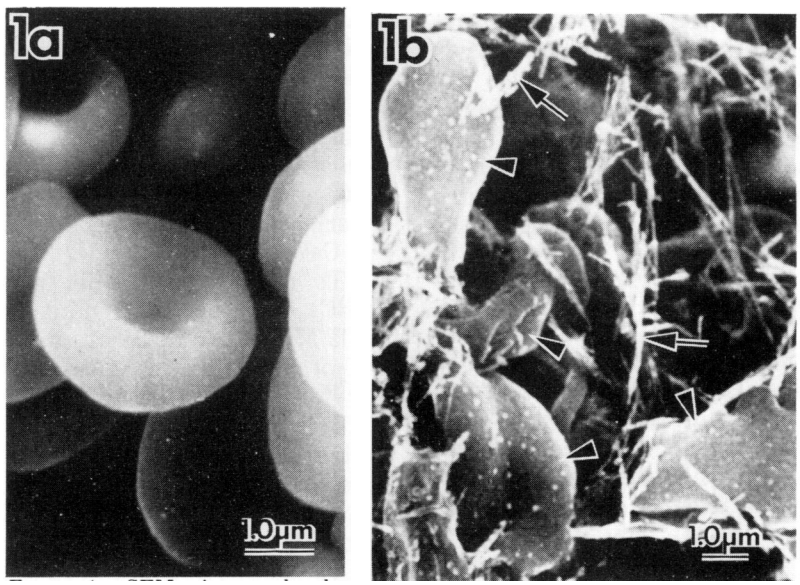


FIGURE 1. SEM micrographs showing (*a*) normal, biconcave RBCs; treatment of RBCs (arrowheads) with chrysotile asbestos (arrows) rapidly causes cell deformation.

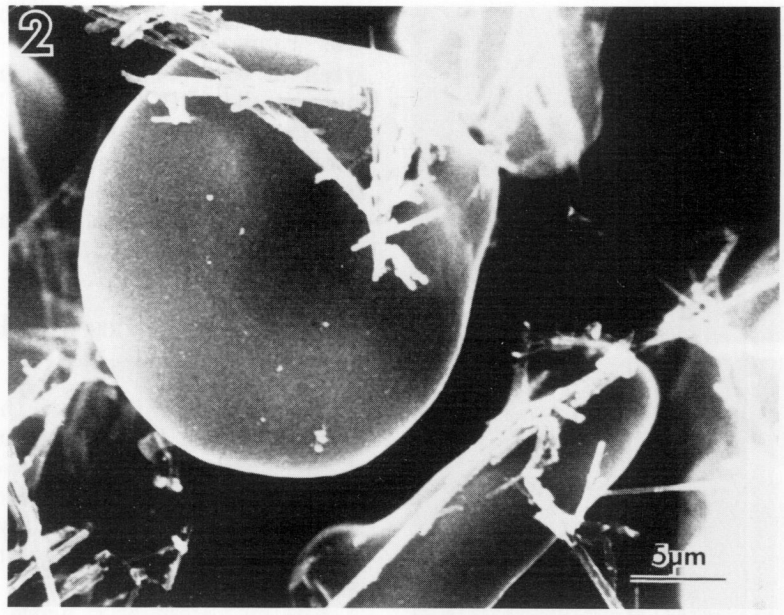
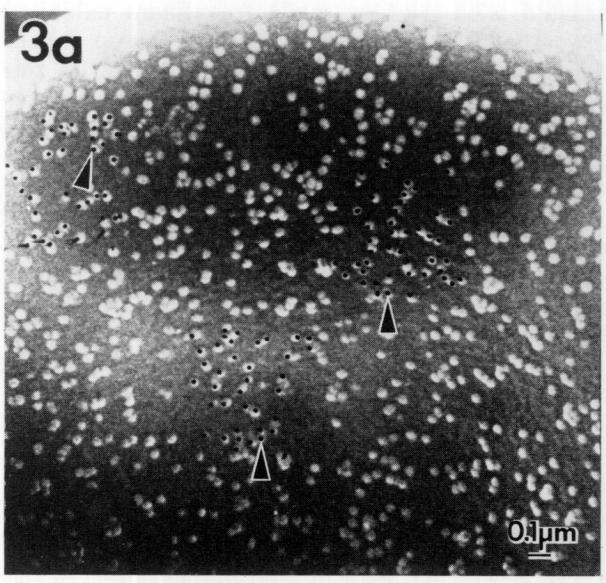


FIGURE 2. Pretreatment of RBCs with neuraminidase protected the cells against the distorting effects of asbestos. The biconcave morphology of these two cells is preserved in spite of large numbers of asbestos fibers.



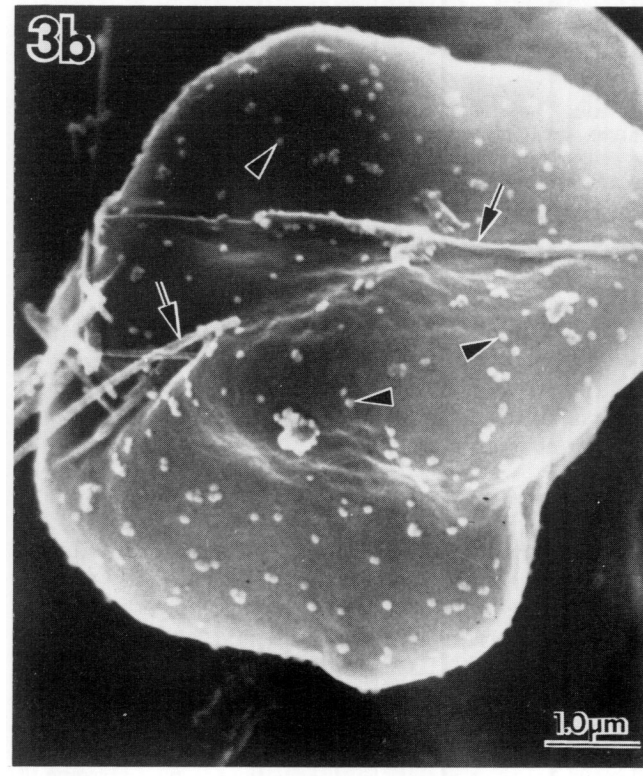


FIGURE 3. Micrograph of (*a*) normal untreated RBC labeled with Au-WGA and (*b*) asbestos-treated RBCs. Note the even distribution of the gold spheres across the surface of the normal cell. The dots on many of the spheres (arrowheads) are the result of our random-field counting technique for determining the number of spheres per unit area of cell surface. Asbestos fibers (arrows) bound to this cell (*b*) have caused a clear change in its shape. There is a reduction in the number of gold spheres (arrowheads) per unit area of cell surface.

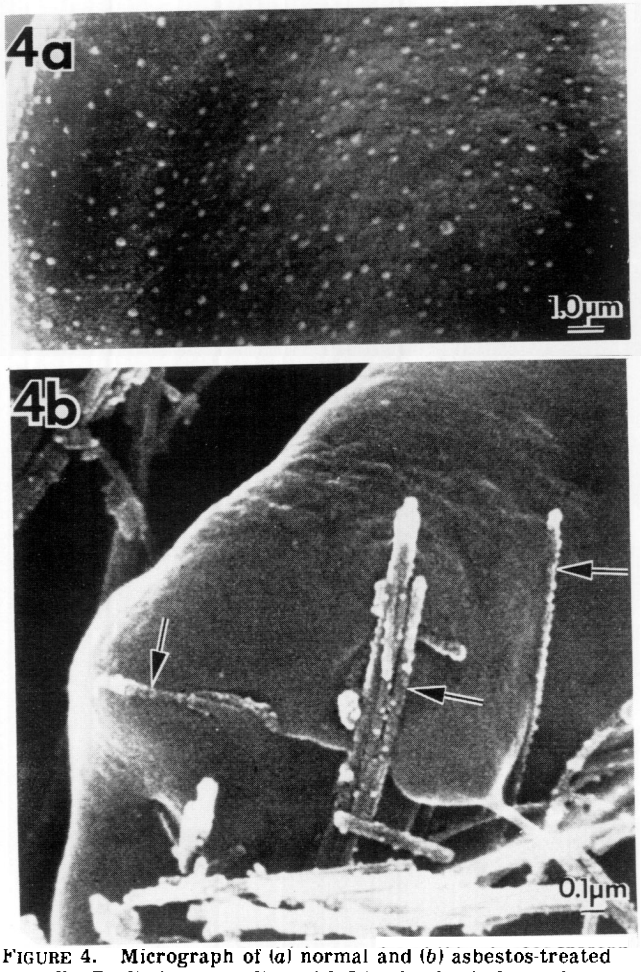


FIGURE 4. Micrograph of (*a*) normal and (*b*) asbestos-treated cells. Preliminary studies with Limulus lectin have shown an even distribution across the surface of normal cells (*a*). The asbestos-distorted cell (*b*) is essentially devoid of the Limulus label, although several fibers embedded in the cell membrane (arrows) appear to be encrusted with what may be the Au-Lim conjugate.

interaction with sialoglycoproteins (*13,14*). Our results are consistent with this view in that RBCs were labeled evenly with Au-WGA before and after treatment with silica.

Intracellular Na^+:K^+ Ratios

The relevance of RBC deformation to the actual events which produce hemolysis is not known. It has been proposed that asbestos-induced redistribution of membrane glycoproteins could lead to alterations of Na^+ and K^+ exchange across cell membranes (*1,14*). We have addressed this issue by determining the intracellular Na^+:K^+ ratios in normal and asbestos-treated RBCs. The mean intracellular Na^+:K^+ ratio in normal appearing untreated cells was 0.56 (range = 0.30-0.85). Cells analyzed from 5 to 15 min after asbestos treatment exhibited a mean ratio of 1.28 (range = 0.82-2.57). Over 120 cells were analyzed in three separate experiments. These data suggest that RBCs distorted by asbestos rapidly lose the ability to balance Na^+ and K^+ ions. Normal ion flux may be adversely affected by redistribution of cell-surface glycoproteins (*1*) and concomitant cell distortion. Anomalies of ion transport are known to result in lysis of RBCs (*15*).

Preliminary Studies with Lectin from *Limulus polyphemus*

A lectin separated from the hemolymph of the horseshoe crab *Limulus polyphemus* is specific for *n*-acetylneuraminic (sialic) acid (*16*). The Limulus protein conjugated to colloidol gold spheres (Au-Lim) was used to further study RBC membrane glycoproteins before and after treatment with asbestos.

Au-Lim conjugates were distributed evenly across the surfaces of normal RBCs (Fig. 4*a*). Preliminary observations of asbestos-treated cells suggested that regions of the cell adjacent to membrane-bound fibers were essentially devoid of the Au-Lim label (Fig. 4*b*). In addition, asbestos fibers embedded in RBC membranes were heavily encrusted with Au-Lim, while free fibers exhibited few attached gold spheres (Fig. 4*b*). These experiments have not been adequately controlled at this point in time, and it is not possible to draw firm conclusions. However, these observations again support the concept of an asbestos-sialic acid interaction which correlates with cell deformation.

Our studies, like those of Sykes et al. (*14*), lend supporting evidence to the concept of asbestos-induced RBC injury mediated through interactions with membrane glycoproteins (*1*). Whether or not these mechanisms are operative in pulmonary cells affected by asbestos is the subject of ongoing studies.

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