Environmental Health Perspectives
Vol. 51, pp. 23-25, 1983

Studies Using Lectins to Determine Mineral Interactions with Cellular Membranes

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Chrysotile asbestos interacts with mucin-secreting cells of tracheal organ cultures, causing an increase in secretion of mucin into the culture medium. This response occurs in the absence of obvious morphologic damage to tracheal epithelial cells. We speculated that asbestos-induced hypersecretion was regulated by the interaction of fibers with specific carbohydrate residues on the cell surface. To test this hypothesis, lectins, i.e., proteins with a high affinity for mono- and oligosaccharides on the plasma membrane, were added to tissues 30 min before addition of chrysotile. Secretion of mucin into the medium was then determined over a 2-hr period by using incorporation of ^{3}H-glucosamine. Blocking of α-D-mannose and α-D-glucose residues inhibited chrysotile-induced hypersecretion ($p < 0.05$), whereas lectins blocking residues of α-D-*N*-acetylgalactosamine, β-D-*N*-acetylglucosamine, α-L-fucose and sialic acids were ineffective. Preincubation of cultures with carboxypeptidase A or phospholipase A_2, but not with neuraminidase, diminished mucin secretion caused by chrysotile. To determine if the positive surface charge of chrysotile was important in interaction with mucin cells, we examined comparatively the effects of various polycations (cationic ferritin, polylysine, DEAE-dextran) and chrysotile after leaching of fibers to remove Mg^{2+}. Although use of polycations enhanced secretion of mucin, effects were not as striking as those observed with chrysotile. In contrast, leached chrysotile failed to elicit a hypersecretory response. These results suggest the interaction of a positively charged component (presumably Mg^{2+}) of chrysotile with glycolipids and glycoproteins containing terminal residues of α-D-mannose or α-D-glucose.

Introduction

We and others (*1-3*) have used hemolysis and scanning electron microscopy (SEM) as tools to investigate the interaction of chrysotile asbestos with red blood cells (RBC). Our results have suggested the binding of the fiber with sialic acid on the surface of the cell. Because the external membrane of the RBC contains a variety of unique glycoproteins including glycophorin, its major sialoprotein, we questioned whether or not these results could be extrapolated to the interaction of asbestos with mammalian cells, specifically epithelial cells of the respiratory tract. Using a novel approach reported recently from this laboratory (*4*), we examined the capacity of different lectins, i.e., proteins binding to selected carbohydrate residues on the plasmalemma, to block chrysotile-induced secretion of mucin by tracheal epithelial cells.

Based on our previous observations which show an association of fibers with nonciliated superficial cells of the tracheal mucosa (*5*), we speculated that a specific interaction between chrysotile and the membrane of the mucin cell triggered hypersecretion.

Methods

Lectins

Concanavalin A (Con A, specific for α-D-mannose, α-D-glucose), *Limulus polyphemus* agglutinin (LPA, sialic acids), *Dolichos biflorus* agglutinin (DBA), α-D-*N*-acetylgalactosamine), *Ulex europaeus* agglutinin (UEA, α-L-fucose) and wheat germ agglutinin (WGA, β-D-*N*-acetylglucosamine) were obtained from Bethesda Research Laboratories, Inc., Gaithersburg, MD). The activity of each lectin was titered and

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determined by hemagglutination before addition to tracheal explants.

Tracheal Organ Cultures

Tracheal explants from male pathogen-free, 6-week-old Fischer rats (Kingston, NY) were prepared and maintained in organ culture as described by Mossman and Craighead (*6*). The tissues were incubated for 18 hr in medium containing 15 μCi/mL ^{3}H-glucosamine HCl (specific activity 5.15 Ci/mmole, New England Nuclear, Boston, MA). After washing with Hanks' balanced salt solution (HBSS) to remove unincorporated label, the explants were transferred to 35 mm Petri dishes (four per dish), and incubated for 5 hr (baseline period) in 0.5 mL fresh medium at 37°C. Medium containing secreted mucin was collected at the end of the baseline period and saved for assay. At this time, specific lectins were added in phosphate-buffered saline (PBS) (pH 7.4) containing 10 *mM* CaCl. After 30 min at 37°C, lectins (20 and 200 μg/mL medium) were removed, tissues were rinsed in HBSS, and chrysotile asbestos (UICC reference sample, 0.4 mg/mL) was added for a 2-hr experimental period. Secretion of mucin into the medium during both baseline and experimental periods was assayed using trichloroacetic acid for precipitation of glycoproteins and scintillation spectrometry of the filtered precipitate (*7*). After fixation in Bouin's solution, explants were then prepared for histology and stained by the alcian blue (pH 2.5)-periodic acid Schiff technique to detect glycoproteins (*8*).

In some experiments, tissues were incubated with neuraminidase (3 mg/mL), phospholipase A_2 (40 and 100 μg/mL) or carboxypeptidase A (40 and 100 μg/mL) (all enzymes from Sigma Chemical Co., St. Louis, MO) for 30 min in PBS prior to the addition of chrysotile. These enzymes cleave sialic acids, phospholipids and peptides bearing a free α-carboxyl group, respectively, from the surfaces of cells.

Polycations and Leached Chrysotile

Because exposure to crocidolite, a slightly negatively charged asbestos, does not elicit hypersecretion (*4*), we speculated that the positive surface charge of chrysotile was important in its interaction with mucin cells. To test this hypothesis, we incubated chrysotile for 1 week in 1 *N* HCl, a process removing Mg^{2+} and the positive charge of the fiber (*2*). In addition, we tested various cationic substances (i.e., DEAE-Dextran, 50 and 200 μg/mL; polylysine, 20 and 200 μg/mL; and cationic ferritin, 1 and 10 μg/mL; all from Sigma Chemical Co., St. Louis, MO) for their ability to stimulate mucin secretion when added in medium for a 2-hr experimental period. UICC chrysotile (04. mg/mL) was used as a positive control in these studies.

Results

After addition to explants, chrysotile caused an increased secretion of mucin that could be blocked with use of Con A, a lectin binding to α-D-mannose and α-D-glucose (Table 1). In contrast, lectins blocking residues of α-D-*N*-acetylgalactosamine, β-D-*N*-acetylglucosamine, α-L-fucose and sialic acids were ineffective. Lectins neither diminished mucin secretion in control cultures nor induced morphologic alterations in tissues.

The prior addition of phospholipase A_2 and carboxypeptidase A inhibited chrysotile-induced, but not normal, mucin secretion (Table 2). These effects appeared dose-dependent with maximum inhibition occurring at 100 g/mL of enzyme. Alternatively, use of neuraminidase did not cause a decrease in mucin secretion either in control or asbestos-exposed cultures. Amounts of the three enzymes used in our studies appeared nontoxic to tracheal epithelial cells.

Table 1. Effects of lectins on chrysotile-induced mucin secretion.[a]

	% of amounts observed in untreated cultures
Chrysotile	168 ± 10 (*N* = 30[b])
Chrysotile + Ulex	198 ± 20 (*N* = 5)
Chrysotile + WGA	185 ± 20 (*N* = 10)
Chrysotile + DBA	165 ± 5 (*N* = 10)
Chrysotile + LPA	178 ± 15 (*N* = 10)
Chrysotile + ConA	130 ± 5* (*N* = 25)

[a]Lectins added at 200 μg/mL medium.
[b]Number of dishes.
*Different from group 1 at $p < 0.05$ level of significance.

Table 2. Chrysotile-induced mucin secretion after addition of carboxypeptidase A, phospholipase A_2 and neuraminidase to rat tracheal epithelium *in vitro*.

	% of amounts observed in untreated cultures[a]
Chrysotile	405 ± 36
Carboxypeptidase A (100 μg/mL)	81 ± 10
Carboxypeptidase A (40 μg/mL) + chrysotile	399 ± 32
Carboxypeptidase A (100 μg/mL) + chrysotile	281 ± 20*
Phospholipase A_2 (100 μg/mL)	90 ± 12
Phospholipase A_2 (40 μg/mL) + chrysotile	320 ± 36
Phospholipase A_2 (100 μg/mL) + chrysotile	300 ± 12*
Neuraminidase (3 mg/mL)	81 ± 11
Neuraminidase (3 mg/mL) + chrysotile	400 ± 15

[a]*N* = 5 dishes per group
*Different from group 1 at $p < 0.05$ level of significance.

Table 3. Mucin secretion by rat tracheal epithelium after exposure to leached chrysotile and cationic substances.

	% of amounts observed in untreated cultures[a]
UICC chrysotile	383 ± 44
Chrysotile leached with 1 *N* HCl	84 ± 9
DEAE-dextran (50 μg/mL)	119 ± 15
DEAE-dextran (200 μg/mL)	289 ± 44
Polylysine (20 μg/mL)	283 ± 15
Polylysine (200 μg/mL)	231 ± 22
Cationic ferritin (1 μg/mL)	136 ± 5
Cationic ferritin (10 μg/mL)	134 ± 6

[a]*N* = 5 dishes per group.

Various polycations stimulated mucin secretion in comparison to untreated cultures, although to varying degrees (Table 3). None produced as striking an effect as chrysotile. In contrast, leached chrysotile did not elicit a hypersecretory response.

Discussion

The mechanisms of chrysotile-induced cell damage have been explored by a number of investigators using both mammalian cells and RBC. In both systems, the positive charge of the fiber appears intrinsic to interaction with cellular membranes. For example, both hemolytic and cytotoxic changes are prevented if the surfaces of fibers are altered by leaching (*2*) or adsorption of serum proteins (*9*). Our results support these observations and suggest that hypersecretion occurs when a positively charged component (presumably Mg^{2+}) of chrysotile interacts with the surface of mucin-secreting cells. Whereas leached chrysotile does not stimulate secretion, various cationic substances do.

We explored the use of lectins to define membrane sites involved in mineral interaction. Results indicate that chrysotile interacts with a surface receptor of α-D-mannose or α-D-glucose on the tracheal epithelial cell. Because preaddition of either carboxypeptidase A or phospholipase A_2 to cultures ameliorates chrysotile-induced secretion, one might speculate that the dust interacts with both glycoproteins and glycolipids on the plasma membrane. Our observations are supported by studies of Jaurand and colleagues (*10*). Using photoelectron spectrometry, these investigators showed the adsorption by chrysotile of both protein and phospholipid components of RBC membranes.

Previous experiments by Harington and colleagues (*1*) and our laboratory (*11*) implicate the interaction of chrysotile with sialic acids on the surface of the RBC. After incubation of RBC with neuraminidase, an enzyme cleaving sialic acid from the cell membrane, chrysotile-induced hemolysis and distortion of RBC membranes are prevented. In contrast, pretreatment of tracheal epithelial cells with neuraminidase does not prevent hypersecretion caused by chrysotile. Thus, chrysotile appears to interact with different membrane sites on various cell types. The use of lectins to define surface components important in mineral-induced cellular responses is a novel approach and one applicable to defining the interaction of a variety of toxic substances with plasma membranes.

This work was supported by Grant #00888 from NIOSH.

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