Alveolar Cell Stretching in the Presence of Fibrous Particles Induces Interleukin-8 Responses

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Inhalation of fibrous particulates is strongly associated with lung injury, but the molecular and cellular mechanisms that could explain the fiber-induced pathogenesis are not fully understood. We hypothesized that the physical stress exerted on the alveolar epithelium by the deposited fibers is greatly enhanced by the tidal cyclic motion of the epithelial cells that is associated with breathing, and that this initial mechanical interaction triggers a subsequent cell response. To test this hypothesis, we developed a dynamic model of fiber-induced cell injury using a cell-stretcher device. We exposed a cyclically stretched monolayer of the human alveolar epithelial cell line A549 to glass or crocidolite asbestos fibers for 8 h and then measured the production of the proinflammatory cytokine interleukin (IL)-8 as a readout of fiber-induced cell injury. Cyclic stretching significantly increased IL-8 production in the fiber-treated cultures, suggesting that the physical stress on the cells caused by the fibers was indeed enhanced by the motion. Coating of the asbestos fibers with fibronectin, a glycoprotein abundant in the alveolar lining fluid, further increased the fiber-induced cell response when the cells were cyclically stretched. This response was, however, significantly reduced by introducing into the culture medium, before fiber treatment, soluble RGD (Arg–Gly–Asp)-containing peptides, which specifically block binding to integrin receptors upon RGD attachment. These results suggested that adhesive interactions between protein-coated fibers and cell surface molecules are involved in the fiber-induced pathogenic process. Our novel findings indicate the importance of physical insults in fiber-induced cell stress, and bring to the forefront the need to study the mechanisms involved in this process. **Tsuda, A., B. K. Stringer, S. M. Mijailovich, R. A. Rogers, K. Hamada, and M. L. Gray. 1999. Alveolar cell stretching in the presence of fibrous particles induces interleukin-8 responses. Am. J. Respir. Cell Mol. Biol. 21:455–462.**

Exposure to fibrous particulates is strongly associated with lung injury, but the molecular and cellular mechanisms responsible for the fiber-induced pathogenesis are not fully understood (1, 2). When inhaled fibers deposit on the acinar walls, they form physical and chemical contacts with the alveolar epithelial cells (3, 4). The role of fiber surface chemistry in fiber-induced lung injury, and the biochemical pathways involved in this process, have been extensively studied (5, 6). The effects of physical contact between fibers and the epithelial cell, however, have not been thoroughly investigated. Donaldson and colleagues (7) found that long amosite asbestos fibers caused a rapid detachment of alveolar epithelial cells in tissue culture, whereas short fibers did not. They further demonstrated that the cell detachment was not caused by oxidative stress (the most commonly studied biochemical consequence of exposure to asbestos fiber), since it could not be ameliorated by scavengers of active oxygen species. These results suggest that the fiber length-dependent cell detachment is likely to be a manifestation of the cell response to physical stimuli. Boylan and coworkers (8) recently reported that coating crocidolite asbestos with the adhesive protein vitronectin enhances fiber internalization by cultured mesothelial cells via the $\alpha_v\beta_5$ integrin receptor. This finding suggests that fibers coated with protein may undergo a receptor-mediated interaction with the cell, leading to a change in the cell's behavior.

In vitro studies on fiber–cell interactions are usually performed in a static cell culture system. *In vivo*, however,

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Abbreviations: enzyme-linked immunosorbent assay, ELISA; interleukin, IL; manmade vitreous fiber, MMVF; arginine–glycine–aspartic acid, RGD; tumor necrosis factor-α, TNF-α.

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the lung is in a vital cyclic motion associated with tidal breathing. We hypothesized that physical stimuli exerted on cells by fibers may be greatly enhanced by this cyclic motion—a factor entirely ignored in current *in vitro* studies—and may trigger a subsequent cell response. To test this idea, we developed a dynamic model of fiber-induced cell injury, using a novel cell-stretcher device. This device produces rhythmic stretching of a monolayer of cultured cells with physiologically relevant tidal breathing conditions. We used the human alveolar epithelial tumor cell line A549 on the cell-stretcher device, and exposed the cell monolayer to either crocidolite asbestos or glass fibers. After stretching and simultaneous exposure of cells to fibers, we measured the production of the proinflammatory cytokine interleukin (IL)-8 as a readout of fiber-induced cell stress responses. We found that cyclic stretching significantly increased IL-8 induction in the fiber-treated cultures, suggesting that the physical stress of the cell by fibers was indeed enhanced by the stretching motion. Coating of the fibers with fibronectin, a glycoprotein abundantly available in the alveolar lining fluid, further increased the cyclically stretched cells' response (as reflected by IL-8 production) to asbestos fiber treatment, and this effect was inhibited by RGD (Arg–Gly–Asp)-containing peptides, which are known to specifically block binding to integrin receptors recognizing the RGD tripeptide sequence (9). This finding suggests that adhesive interactions between protein-coated fibers and cell surface determinants may also be involved in the pathogenesis of fiber-induced lung injury.

Materials and Methods

Cells and Cell Culture

Cells of human alveolar epithelial tumor cell line A549 were obtained from the American Type Culture Collection (Rockville, MD). The cells were cultured on either fibronectin-coated or collagen type I-coated silastic membranes of a stretching device (*see the following discussion*) in RPMI 1640/10% fetal calf serum supplemented with penicillin, streptomycin (100 U/ml each) and L-glutamine (2 mM) at 37°C in a 5% CO_2 atmosphere.

Cell-Stretching Device

Detailed design criteria for the custom-made cell-stretching device (Z-development, Cambridge, MA) used in the experiments were previously described (10). Briefly, the cell stretcher consisted of: (*1*) four identical culture wells (7.68 cm diameter), containing as the deformable culture surface a stretchable membrane made of high-strength silicone elastomer (Specialty MFG Inc., Saginaw, MI); and (*2*) a drive system simultaneously providing identical deformation profiles to four culture membranes. The device can generate biaxially uniform and isotropic strain (equivalent radial and circumferential strain over the entire culture membrane) at stretching frequencies ranging from 0.01 Hz to 10 Hz.

Fibers, Fiber Preparation, and Coating

Needle-shaped crocidolite asbestos distributed by the International Union Against Cancer (UICC), was a gift from J. J. Godleski of the Department of Pathology, Harvard Medical School, Boston, MA. The length distribution of UICC crocidolite asbestos was previously reported by Timbrell (11); about 40% of the asbestos particles in a sample are shorter than 1 μm (as measured electron microscopically), 40% range between 1 to 8 μm, and 20% are longer than 8 μm (as measured with optical microscopy). Glass fibers (manmade vitreous fiber [MMVF-10]) were provided by Schuller International Inc., Littleton, CO. The glass fibers have been previously characterized and found to have a mean length of 22.6 ± 18.6 μm (mean ± SD) (range: 1.8–74 μm) and a mean diameter of 1.3 ± 0.85 μm (range: 0.1–4.2 μm). The chemical composition of MMVF-10 glass fiber was reported by the manufacturer and described in detail in our previous paper (12).

Both crocidolite asbestos and glass fibers were suspended in phosphate-buffered saline (PBS) at a concentration of 2 mg/ml, sterilized overnight under UV light, and stored at 4°C. Before use, the fibers were washed and resuspended in cell culture medium, warmed to 37°C, and vortexed to ensure a uniform suspension.

For coating the fibers with proteins, 1 ml fibronectin (Sigma, St. Louis, MO) solution (1 mg/ml in H_2O) was added to 1 ml of fiber suspension, and this mixture was incubated for 2 h at room temperature with occasional shaking. The fibers were washed twice with PBS and resuspended in cell culture medium at 1 mg/ml concentration. Immediately before use, the fiber suspension was diluted to final concentration (500 μl/13 ml medium for each plate). In some experiments, after incubating with fibronectin, the fibers were washed twice and then incubated in bovine serum albumin (BSA) (0.1% in H_2O) for 10 min to block nonspecific binding of serum protein (8, 13). The fibers were then washed twice with PBS and resuspended in cell culture medium.

To confirm and quantify the adsorption of fibronectin on fiber surfaces, the fibronectin-coated fibers were visualized by incubation with mouse antihuman IgG_1 to fibronectin (Zymed Laboratories, South San Francisco, CA), which was followed by treatment with goat antimouse Ig labeled with Texas red (Sigma). The fluorescence intensity on the fiber surfaces was analyzed with a Leica confocal microscope (Sarastro 2000; Molecular Dynamics, Sunnyvale, CA).

Fiber Treatment and Cell Stretching

Confluent monolayers of A549 cells were treated with fibers (asbestos or glass, fibronectin-coated and uncoated) at a dose of 500 μg/plate (10 μg/cm^2). The fibers were allowed to settle in the culture medium for 1 h[1] and to make physical contact with the cell surface before cell stretching. Control cells receiving no fibers were incubated with medium or medium containing fibronectin (0–10 μg/ml) alone.

[1] Using fiber kinetics equation (Eq. 12.11 on page 39 in *The Mechanics of Aerosols* by N.A. Fuch, 1963 [Ref. 14]), it was estimated that approximately 1 h is necessary for most of the fibers (length > 0.1 μm, aspect ratio of 3–40) to sediment through the culture medium of 13 ml (depth of 0.26 cm) and to make physical contact with the cell surface. This volume (13 ml) of culture medium was required to ensure that the cells would not be exposed to the air during cyclic stretching.

In cell-stretching experiments, the cells were cyclically stretched with a strain of 5% at 10 times per minute for 8 h. Static experiments were done at the same time under identical culture conditions but without stretching. After 8 h of fiber exposure (stretched or static), the viability of the cells as measured with a standard trypan-blue exclusion assay was generally greater than 90%. Cell culture supernatant samples were collected for IL-8 assays.

IL-8 responses to nonparticle stimuli of IL-8 production (18) were tested under both static and stretched cell culture conditions, using tumor necrosis factor-α (TNF-α) (Sigma) at various concentrations as a stimulus (0–20 ng/ml).

RGD Blocking

A549 cells were grown on a collagen type I-coated stretchable silicone membrane so that the cells adhered to the membrane in an RGD-independent manner (15). Soluble RGD-containing peptides (GRGDS; Sigma) were added at a concentration of 80 μg/ml to the cell culture at 15–30 min (16, 17) before exposure of the cells to fibronectin-coated asbestos fibers (10 $\mu g/cm^2$). In the RGD blocking experiments, the fiber samples were treated with BSA after fibronectin coating (*see the section on* FIBER, FIBER PREPARATION, AND COATING) to prevent nonspecific binding of other serum proteins present in the tissue culture medium.

Enzyme-Linked Immunosorbent Assay for IL-8

IL-8 measurements were made in duplicate in 96-well plates as previously described (18). Briefly, a mouse monoclonal IgG_1 antibody to human IL-8 (R&D Systems, Minneapolis, MN), was used at 50 ng/well in PBS as the capture antibody. The wells were blocked with 1% BSA in PBS–Tween, and serial 2-fold dilutions of the samples were then applied. For detection, a rabbit polyclonal anti-human IL-8 antibody (Endogen, Cambridge, MA) was used with horseradish peroxidase-conjugated polyclonal goat antirabbit Ig antibody (Sigma). The reaction was visualized with tetramethylbenzidine (TMB) tablets as substrate, and the color intensity was read with a Vmax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) at 450 nm. Serial dilutions of recombinant human IL-8 (R&D Systems) were used as a standard. In the RGD blocking experiments, an ELISA kit for IL-8 (R&D Systems) was used according to the manufacturer's instructions.

Statistical Analysis

The IL-8 concentrations produced by the stretched and static cultures were compared with the paired Student's *t* test, using commercial software (SigmaPlot, version 3; Jandel, San Rafael, CA). Differences were considered significant at $P < 0.05$.

Results

We measured the induction of IL-8 in the culture medium of A549 cells treated with different fibers with or without cyclic stretching. IL-8 is a major neutrophil chemotactic factor in the lung, and plays a central role in the process of pulmonary inflammation (19). Although alveolar macrophages (AM) are known to be major producers of IL-8, mesothelial cells and alveolar epithelial cells (A549 cell line) have also been shown to synthesize IL-8 in response to various stimuli (20), including asbestos fibers (21, 22).

Cyclical Stretching of Alveolar Epithelial Cells *In Vitro* Does Not Induce IL-8

In vivo, alveolar cells are subjected to cyclic stretching associated with tidal breathing. Using a cell stretcher device, we tested the response of an A549 cell monolayer to *in vitro* cyclic stretching with physiologic parameters (a strain of 5% at 10 times per minute) for 8 h. Under these stretching conditions, the levels of IL-8 produced by the cells did not exceed the background levels produced by "static" A549 cell cultures (cells grown on the identical culture plates with silastic membrane in the same experiments) (Figure 1). Both static and cyclically stretched A549 cell cultures had more than 90% cell viability, indicating that growth on silastic membrane and cell stretching did not interfere with essential cell functions.

Induction of IL-8 by Fiber Treatment Is Largely Enhanced by Cyclic Stretching

Treatment of static cells with glass fibers (10 $\mu g/cm^2$) resulted in a slight but not statistically significant increase in IL-8 production (Figure 2) over the background levels (Figure 1). Treatment with glass fibers combined with cell stretching, however, resulted in a statistically significant ($P = 0.0006$) increase in IL-8 induction, suggesting that the mechanical interaction between the fibers and cells was enhanced by the cyclic motion of the cells and triggered an inflammatory cell response.

Treatment of static cells with crocidolite asbestos fibers (10 $\mu g/cm^2$) resulted in IL-8 secretion into the medium. More importantly, the induction of IL-8 secretion was again significantly ($P = 0.015$) enhanced by cyclic stretching of asbestos-treated cells (Figure 2).

Fibronectin Coating of Fibers Enhances Their Ability to Induce IL-8 Responses in Cyclically Stretched Cells

In the next series of experiments, we tested cell responses induced by fibers coated with fibronectin, one of the pro-

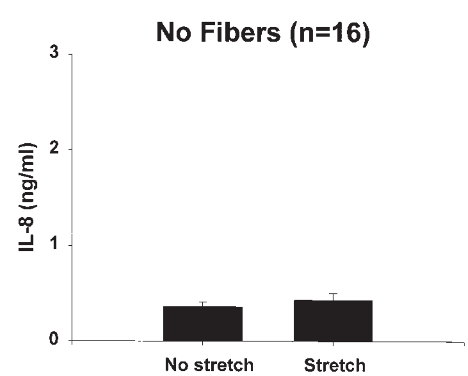

Figure 1. IL-8 production in static and cyclically stretched cultures of human A549 alveolar epithelial cells in the absence of fibers. Stretching conditions consisted of a strain of 5% at 10 times per minute for 8 h. Values are means ± SEM, $n = 16$.

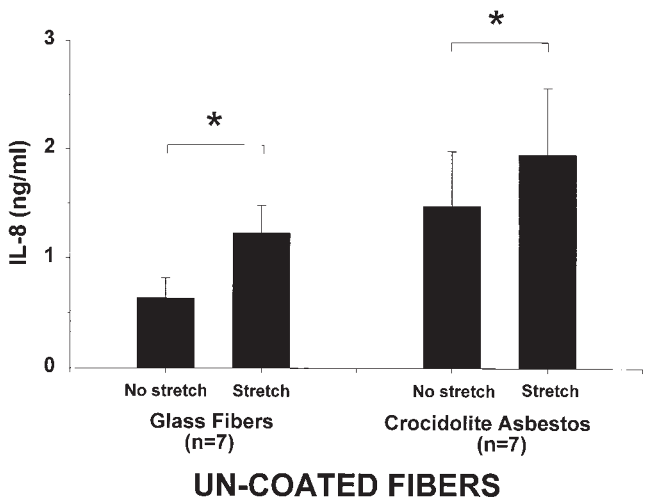

Figure 2. IL-8 responses to uncoated fibers (glass or crocidolite asbestos fibers) at 10 μg/cm^2 in static and cyclically stretched cultures of A549 cells. Stretching conditions consisted of a strain of 5% at 10 times per minute for 8 h . Values are means ± SEM (*$P < 0.05$, paired t test).

TABLE 1

*Fibronectin-coating on crocidolite asbestos and glass fibers after various numbers of washings with phosphate-buffered saline**

No. of Washings	Crocidolite Asbestos Fibers	Glass Fibers (MMVF-10)
2	131.95	139.44
5	131.96	105.44
10	132.61	92.93

Definition of abbreviation: MMVF = manmade vitreous fiber.

*Coating of fibers with fibronectin was visualized by immunofluorescence staining, and the fluorescence intensity (scaled from 0 to 255) per unit fiber surface (μm^2) was analyzed with a Leica confocal microscope. A mean of approximately 100 samples was analyzed for each wash for both asbestos and glass fibers.

teins abundantly present in alveolar lining fluid. Coating of glass and crocidolite asbestos fibers with fibronectin was first confirmed by immunofluorescence staining with fibronectin-specific reagents (*see* METHODS). Confocal microscopic visualization revealed that both asbestos and glass fibers were coated with fibronectin (*top panels* in Figure 3, shown in pseudocolors). Interestingly, however, although the fluorescence intensity (scaled from 0 to 255) on the asbestos fiber surfaces remained nearly constant regardless of the number of washings of the fibers with PBS, the fluorescence intensity on the glass fiber surfaces gradually decreased with an increasing number of washings (Table 1 and *middle panels* in Figure 3). Surfaces of approximately 100 samples were analyzed for each wash for both asbestos and glass fibers, and the mean fluorescence intensity per unit fiber surface (μm^2) was computed. This suggested that asbestos fibers adsorbed fibronectin more strongly than did glass fibers. The lower panels in Figure 3 are micrographic views of noncoated fiber surfaces, showing negligible background fluorescence (mean fluorescence intensity of 2–23/μm^2) for both types of fibers. It should also be noted that the secondary antibody alone did not stain either fibronectin-coated or uncoated fibers (mean fluorescence intensity less than 10/μm^2, data not shown), suggesting that the observed staining was indeed specific for fibronectin.

Comparison of IL-8 responses of static and stretched A549 cell cultures treated with fibronectin-coated fibers (glass and crocidolite asbestos) indicated that cyclical stretching significantly enhanced IL-8 secretion by the cells exposed to fibronectin-coated fibers ($P = 0.02$ and $P = 0.018$, respectively). This effect was more pronounced in cultures treated with fibronectin-coated crocidolite asbestos fibers (Figure 4). In addition, whereas fibronectin coating greatly enhanced the ability of asbestos fibers to induce IL-8 secretion, it had no appreciable effect on IL-8 induction by glass fibers. This difference is probably explained by the limited ability of glass fibers to retain fibronectin coating (Figure 3). It should be noted that IL-8 was not induced in cells treated with various concentrations (0, 1, 2, and 10 μg/ml) of fibronectin alone (data not shown). Of note is that cyclic stretching of alveolar epithelial cells treated with TNF-α (another known stimulus inducing IL-8 secretion [18, 20]) did not further increase the IL-8 responses of these cells (Figure 5). This observation suggests that the mechanical interaction between the fibronectin-coated fibers and the cells was important in evoking an enhanced response in the cyclically stretched cultures, and that cell stretching *per se* did not facilitate IL-8 responses.

Fibronectin-Coated Fiber-Induced IL-8 Secretion Is Integrin-Mediated

The obtained results suggested that fibronectin-coated asbestos fibers might bind to A549 cell surface determinants through specific integrin-mediated adhesion, and that this interaction, under dynamic conditions, might play a crucial role in evoking subsequent IL-8 responses by the cells. To test this hypothesis, we performed a set of experiments designed to block the fibronectin-binding sites of integrin molecules with soluble RGD peptides. We found that pretreatment of cyclically stretched A549 cells with 80 μg/ml RGD peptides significantly ($P = 0.002$) inhibited fibronectin-coated asbestos-mediated IL-8 production as compared with the results obtained in cultures without RGD pretreatment (70% inhibition of IL-8 secretion: Figure 6; 0.68 ± 0.19 ng/ml [mean ± SEM], and 2.25 ± 0.29 ng/ml, respectively). Pretreatment of static cell cultures with RGD peptides had no significant effect on IL-8 production after exposure of the cultures to fibronectin-coated asbestos (data not shown). It should be noted that RGD treatment in the absence of fibers neither caused a change in cell morphology (data not shown) nor inhibited the IL-8 response of cells to a nonparticle stimulus of IL-8 secretion, TNF-α (Figure 6). The IL-8 response to 10 ng/ml TNF-α in cyclically stretched A549 cell cultures with and without RGD pretreatment was not significantly different (12.2 ± 1.55 ng/ml [mean ± SEM] and 9.8 ± 0.95 ng/ml, respectively).

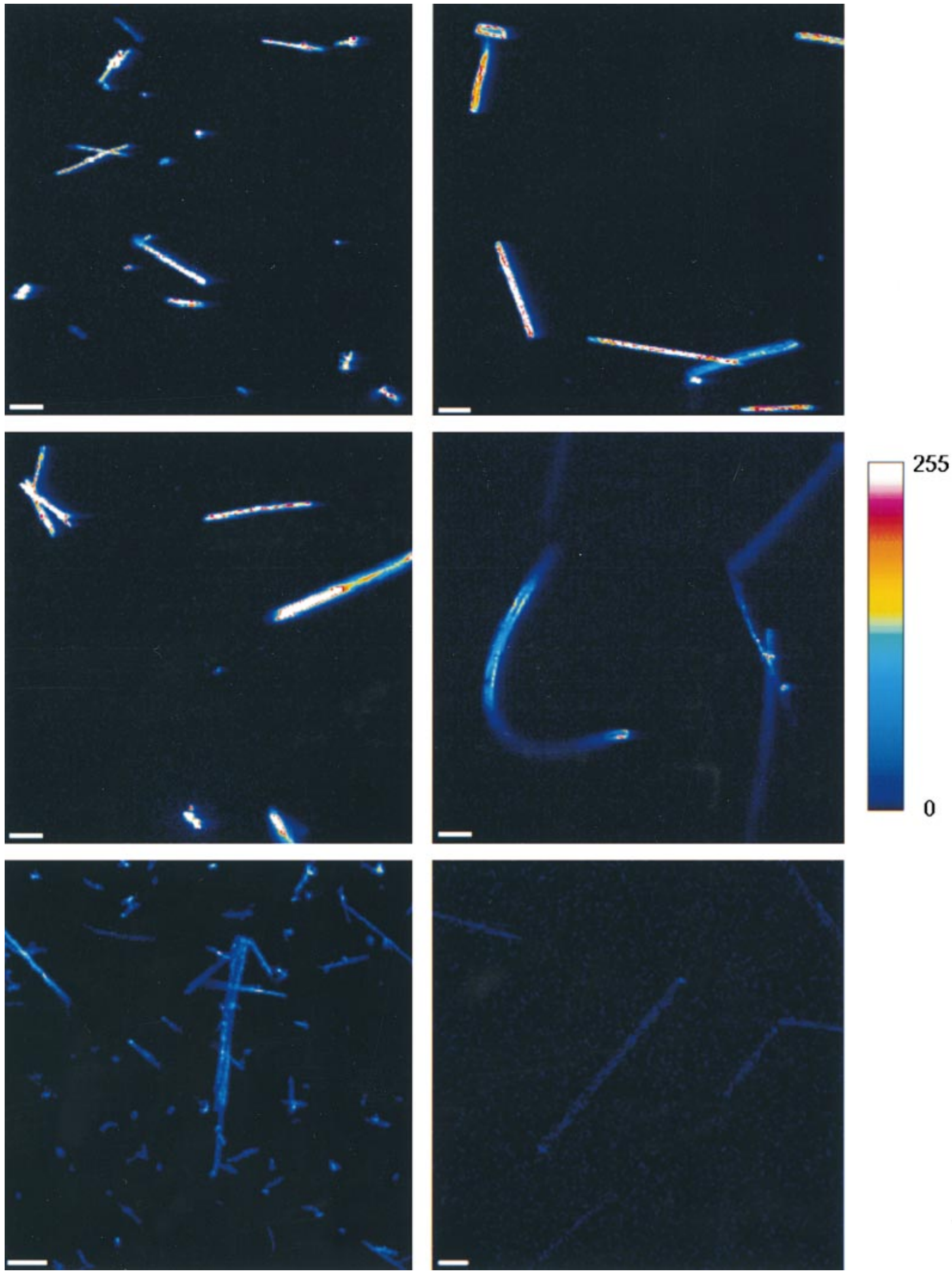

Figure 3. Immunofluorescence visualization of fibronectin-coating on fiber surfaces done with confocal microscope and shown in pseudocolors. The color code of fluorescence intensity (scaled from 0 to 255) is indicated by the color bar on the right. *Top left:* Fibronectin-treated asbestos fibers (2× wash) appear bright white, indicating the presence of fibronectin coating. *Top right:* Fibronectin-treated glass fibers (2× wash) also show fibronectin on the surface. *Middle left:* Fibronectin-treated asbestos fibers washed 10 times still appear bright white, indicating the presence of fibronectin coating after 10 washings. *Middle right:* Fibronectin-treated glass fibers washed 10 times show loss of fluorescence intensity. *Bottom left* and *right:* Uncoated asbestos and glass fibers appear in dark blue, showing negligible background fluorescence. *Bars* show 10 μm.

Dose Response

Dose–response relationships between the fiber concentration and IL-8 production were also evaluated in a set of separate experiments. Although the data points show a wide variation, fibronectin-coated asbestos showed a trend toward inducing increased IL-8 production with increased fiber dosage in both static and stretched A549 cells (Figure 7). Importantly, the stretched-cell cultures treated with fi-

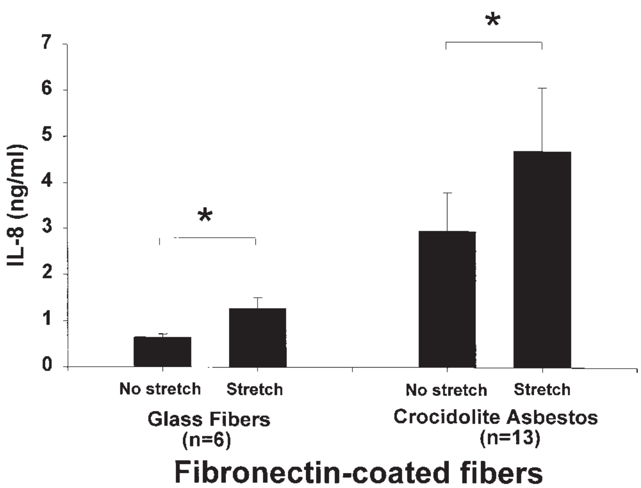

Figure 4. IL-8 responses to fibronectin-coated fibers (glass or crocidolite asbestos fibers) at 10 μg/cm^2 in static and cyclically stretched cultures. Stretching conditions consisted of a strain of 5% at 10 times per minute for 8 h. Values are means ± SEM (*$P <$ 0.05, paired *t* test).

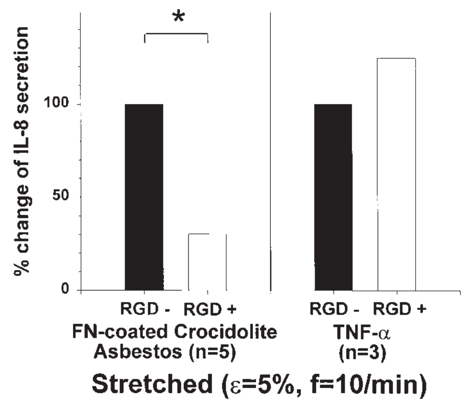

Figure 6. *Left panel:* Percent reduction of IL-8 responses of cyclically stretched A549 cells exposed to fibronectin-coated crocidolite asbestos fibers at 10 μg/cm^2 in the presence (RGD +) or absence (RGD −) of RGD peptides (80 μg/ml). *Right panel:* Percent change in IL-8 secretion in responses to TNF-α (10 ng/ml) in the presence (RGD +) or absence (RGD −) of RGD peptides (80 μg/ml). Stretching conditions consisted of a strain of 5% at 10 times per minute for 8 h. Values are means. (*$P < 0.05$, paired *t* test).

bronectin-coated asbestos consistently exhibited higher IL-8 production than did static cultures.

Discussion

The principal finding of our study was that the fiber-induced inflammatory cytokine (IL-8) response of alveolar epithelial cells was greatly enhanced by cyclic stretching of a monolayer of these cells (Figures 3, 4, and 6). Because this phenomenon was observed not only in cells exposed to asbestos fibers but also with cells treated with chemically inert glass fibers, a physical interaction between the fibers and the cyclically stretched cells was likely to have played an important role in the induction of this response.

Our findings are consistent with the well-recognized but still unexplained notion that one of the important factors in fiber-induced pulmonary pathology is the shape of the particles. In animal models, for instance, Davis and coworkers found that animals exposed to longer fibers (both glass and asbestos) had a much higher incidence of pulmonary inflammation, fibrosis, and lung cancer than did those exposed to shorter fibers (23, 24). Mossmann and her group exposed several different cell types (hamster and rat airway epithelial cells, AM, mesothelial cells, and rat embryo cells) in tissue culture to both crocidolite asbestos fibers and to a nonfibrous analogue of crocidolite, and found that the fibrous particles were much more potent in causing cell damage than were the nonfibrous particles at comparable exposure dosages (25–28). These results suggest that the fiber-length (or -shape)-dependent pathogenesis of fiber-induced lung injury is a manifestation of physical cell injury. The increased pathogenicity of fibrous particles

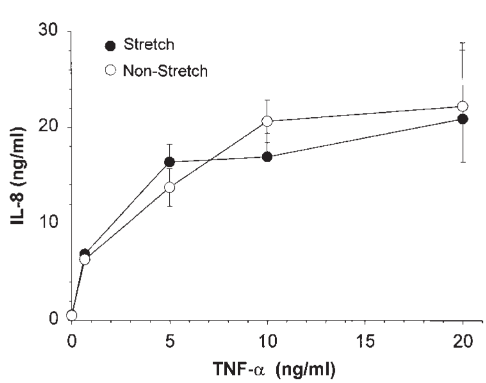

Figure 5. IL-8 responses to various concentrations of TNF-α (0–20 ng/ml) in cyclically stretched (*closed circles*) and static (*open circles*) cultures; $n = 5, 1, 4, 5$, and 4 cultures tested at TNF-α concentrations of 0, 0.67, 5, 10, and 20 ng/ml, respectively. Stretching conditions consisted of a strain of 5% at 10 times per minute for 8 h. Values are means ± SEM.

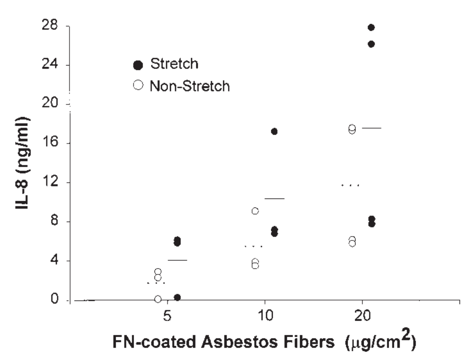

Figure 7. IL-8 dose responses of A549 cells exposed to fibronectin-coated crocidolite asbestos fibers at 5, 10, and 20 μg per unit culture surface (cm^2) in cyclically stretched (*closed circles*) and static (*open circles*) cultures. *Solid* and *dashed lines* show mean values. Stretching conditions consisted of a strain of 5% at 10 times per minute for 8 h.

may be explained by a mismatch in strains between the fibers and the cells under dynamic conditions (29). Physical contact of rigid fibers on the surface of lung cells may significantly restrict the cyclic motion of the alveolar epithelium (30). Our theoretical analysis shows that the mismatch in strains would result in development on the cell surface of traction that is strongly fiber-length dependent. It awaits future investigation to see whether different shaped particulates of identical chemical composition (e.g., crocidolite versus riebeckite) would produce different cell responses in dynamic experiments, confirming our hypothesis.

When fibers deposit on the acinar walls, they first encounter the alveolar lining fluid before making physical contact with the epithelial cells (4). Because the alveolar lining fluid contains many proteins, such as albumin and fibronectin (31, 32), and the surfaces of many fibers are charged (33–35), these fibers adsorb proteins available in the lining fluid (8, 36, 37). The protein-coated fibers may undergo adhesive interactions with cell surface receptors. Boylan and associates (8) demonstrated that vitronectin-coated fibers could bind adhesively with cell surface receptors (the integrins $\alpha_v\beta_5$ and $\alpha_v\beta_3$) on mesothelial cells. It is therefore reasonable to assume that the fibronectin-coated fibers in our experiments could have formed a receptor-mediated adhesive contact with integrins specific to fibronectin, such as integrins $\alpha_v\beta_3$ and $\alpha_3\beta_1$, which are known to be present on the surfaces of A549 alveolar epithelial cells (7, 38). The fact that soluble RGD peptides blocked the induction of IL-8 in our stretched-cell cultures treated with fibronectin-coated asbestos fibers supports this view, since these peptides specifically inhibit the binding of fibronectin to RGD-recognizing sites on integrin receptors. Although integrins were originally thought to be responsible only for anchoring cells to the extracellular matrix (39), they have been recently recognized as major players in the regulation of basic cell functions such as proliferation, differentiation, and apoptosis (40–43). Therefore, attachment of fibers to integrins, and perhaps the triggering of integrin signaling, may be very important in inducing a variety of subsequent cell responses. Furthermore, under conditions of cyclic cell motion, the protein-coated fibers and integrins are likely to be clustered, which could lead to crosslinking of the receptors and/or the repeated attachment and detachment (and reattachment) of ligands to receptors, and these events may amplify the signals generated (17, 44–47).

In summary, our finding of an enhanced fiber-induced proinflammatory cytokine response in cyclically stretched alveolar epithelial cells as compared with cells treated with the same fibers in a static situation suggests that *in vivo*, the vital cyclic motion of the lung may be very important in enhancing the ability of a fiber to mechanically injure the cell or alter cell responses. The dynamic model of rhythmically stretched cells therefore allows the investigation of essential aspects of the fiber-induced pathogenic process.

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