IL-1β decreases the elastic modulus of human tenocytes

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IL-1β decreases the elastic modulus of human tenocytes. J Appl Physiol 101: 189–195, 2006. First published April 20, 2006; doi:10.1152/japplphysiol.01128.2005.—Cellular responses to mechanical stimuli are regulated by interactions with the extracellular matrix, which, in turn, are strongly influenced by the degree of cell stiffness (Young’s modulus). It was hypothesized that a more elastic cell could better withstand the rigors of remodeling and mechanical loading. It was further hypothesized that interleukin-1β (IL-1β) would modulate intracellular cytoskeleton polymerization and regulate cell stiffness. The purpose of this study was to investigate the utility of IL-1β to alter the Young’s modulus of human tenocytes. Young’s modulus is the ratio of the stress to the strain, E = stress/strain = (F/A)/(ΔL/L0), where L0 is the equilibrium length, ΔL is the length change under the applied stress, F is the force applied, and A is the area over which the force is applied. Human tenocytes were incubated with 100 pM recombinant human IL-1β for 5 days. The Young’s modulus was reduced by 27–63%. Actin filaments were disrupted in >75% of IL-1β-treated cells, resulting in a stellate shape. In contrast, immunostaining of α-tubulin showed increased intensity in IL-1β-treated tenocytes. Human tenocytes in IL-1β-treated bioartificial tendons were more tolerant to mechanical loading than were untreated counterparts. These results indicate that IL-1β reduced the Young’s modulus of human tenocytes by disrupting the cytoskeleton and/or downregulating the expression of actin and upregulating the expression of tubulins. The reduction in cell modulus may help cells to survive excessive mechanical loading that may occur in damaged or healing tendons.

interleukin-1β; cell modulus; tendon; actin; tubulin

CELLS IN NATURAL ENVIRONMENTS are subjected to a complex biomechanical environment, including tension, compression, and fluid shear stress. Evidence indicates that mechanical signals play critical roles in cell differentiation, proliferation, tissue development, skeletal maintenance, and recovery postsurgery (7, 8, 44). Mechanical signals are transduced in cells and regulate their responses to chemical stimulation (3, 7, 14). The mechanisms involved in this cross talk between mechanical and chemical signals are still poorly understood (7, 37).

In part, cells transduce mechanical stimuli through deformation of the cytoskeleton (20–24). The deformability of a cell is determined by a number of factors, including residual tensile “prestress” (residual stress) in its cytoskeleton, which is influenced by the stiffness of the matrix, attachment of the cell to the matrix, cell-cell connections, and contractility, which together determine the elastic stiffness [Young’s modulus, the ratio of the stress to the strain, E = stress/strain = (F/A)/(ΔL/L0), where L0 is the equilibrium length, ΔL is the length change under the applied stress, F is the force applied, and A is the area over which the force is applied] of the cell (15, 27). A body of evidence is growing, supporting the idea that the tensile pre-stress in the cytoskeleton affects a cell’s response to mechanical stimulation and therefore to chemical signals (44). Therefore, modulation of cytoskeletal stress may play an important role in tissue development, recovery postsurgery, and in the manufacture of engineered tissues in vitro. Agents, such as interleukin (IL-1β), that can modulate the cell’s cytoskeletal presress state will be important in regulating the phenotype and thus the fabrication of engineered tissues.

IL-1β treatment increases the secretion and expression of metalloproteinases (MMPs)-1, -2, -3, -9, and -13 in tenocytes (3, 10, 37), bone cells (30), and chondrocytes (26). Most of these studies were focused on the degradation of matrix induced by IL-1β. No studies addressed the effects of IL-1β on the Young’s modulus of cells, although recent studies show that IL-1α increases the F-actin content of articular chondrocytes (32). Since IL-1β accelerates the degeneration of matrix, it should also reduce matrix stiffness and cell-matrix attachment. The Young’s modulus of cells may also be altered by IL-1β in response to changes in matrix properties or cell attachment. Cell modulus is believed to be mainly determined by the distribution and structure of the cytoskeleton, which is composed of microfilaments, intermediate filaments, and microtubules (33, 35). Disruption of the cytoskeleton dramatically changed the cell modulus (33, 35, 43). Therefore, we hypothesized that IL-1β treatment would reduce the cell modulus by altering the structure of the cytoskeleton and/or regulating the expression of cytoskeletal proteins. A corollary of this hypothesis was that a cell would increase its elasticity (reduce stiffness), facilitate stretching, and better tolerate mechanical loading. In this study, the effects of IL-1β on the Young’s modulus of human tendon internal fibroblasts (HTIFs) and cell viability under extreme mechanical conditions were investigated.

MATERIALS AND METHODS

Cell culture. HTIFs were isolated after surgery from discarded human tendon tissues, as described previously (5). HTIFs from passages 2 to 4 from three different patients were used in this study (patient 1, 2-yr-old male, flexor digitorum superficialis tendon; patient 2, 84-yr-old female, Dupuytren’s contracture; patient 3, 77-yr-old...
female, flexor carpi radialis tendon). HTIFs were maintained in medium 199 (GIBCO, Grand Island, NY) containing 10% fetal bovine serum (FBS; Hyclone, Logan, UT), 20 mM HEPES (pH 7.2, GIBCO), and 1% penicillin/streptomycin solution (GIBCO). HTIFs were allowed to attach and spread for 24 h before addition of 100 pM recombinant human IL-1β. The serum concentration was reduced from 10 to 2%. Culture medium was changed daily. On day 5 was added), cells were released from the culture dishes with 1% collagenase II (Worthington, Lakewood, NJ) at 37°C for 30 min. The released cells were sedimented and suspended in serum-free medium 199.

**Measurement of Young’s modulus of HTIFs.** The Young’s modulus of at least 15 cells from each group was measured using a micropipette aspiration technique (16, 35). With this technique, real-time measurements of pressure and deformation can be made and, in conjunction with theoretical models, can be used to determine the intrinsic mechanical and volumetric properties of a single cell. In brief, the solution and cells were placed in a chamber that allowed for the entry of a micropipette from the side. Micropipettes were made by drawing out glass capillary tubes (A-M Systems, Carlsborg, WA) with a pipette puller (David Kopf Instruments, Tujunga, CA) and fracturing drawing out glass capillary tubes (A-M Systems, Carlsborg, WA) with intrinsic mechanical and volumetric properties of a single cell. In of at least 15 cells from each group was measured using a micropipette aspiration technique (16, 35). During the application of pressure, video images of cell aspiration into the micropipette were recorded on an S-VHS video cassette recorder at 60 fields/s with a charge-coupled device camera (COHU, San Diego, CA), through a bright-field microscope (Diaphot 300, Nikon, Melville, NY), using a ×40 oil immersion objective (numerical aperture 1.25; Nikon). The applied pressures and times were displayed on a video monitor using a digital multiplexer (Vista Electronics, Ramona, CA) and recorded to videotape. The length of cell projection into the micropipette and the micropipette inner diameter were measured with a video caliper system (resolution ±0.2 μm).

**Staining microfilaments and microtubules.** HTIFs were fixed with 3.7% formaldehyde in phosphate-buffered saline (PBS), pH 7.2, at room temperature for 15 min, permeabilized with 0.1% Triton X-100 in PBS at room temperature for 20 min, and rinsed with PBS twice. Microfilaments (microfilaments) were stained at room temperature for 1 h with rhodamine-phalloidin (Molecular Probes, Eugene, OR, at 4°C dilution in PBS). The cells were then rinsed with PBS twice and mounted on glass slides. Microtubules were stained with anti-α-tubulin and anti-β-tubulin monoclonal antibodies, respectively (Sigma). The permeabilized HTIFs were blocked with PBS containing 5% BSA and 2% goat serum at room temperature for 2 h and washed with PBS twice. Then the cells were incubated with the first antibody (1:1,000 for α-tubulin, 1:200 for β-tubulin, diluted in PBS) at 4°C overnight. After washing with PBS, the cells were incubated with Alexa Fluor 488-conjugated (α-tubulin) or Alexa Fluor 568 (β-tubulin) goat anti-mouse IgG (Molecular Probes; 1:200 diluted in PBS) at room temperature for 2 h. The cells were washed with PBS and mounted on glass slides. Cells were imaged using a LeicaSP2 AOB laser scanning confocal microscope (Leica Microsystems, Exton, PA) with a ×40 oil immersion objective.

**Expression levels of actin and tubulin using quantitative RT-PCR.** Cells were collected on days 1, 3, and 5 after addition of IL-1β. Total RNA was isolated using an RNeasy mini kit (QIAGEN, Valencia, CA), according to the manufacturer’s protocol. Complementary DNA was synthesized with SuperScriptII (Invitrogen, Carlsbad, CA). The expression levels of actin and tubulin were determined by semiquantitative RT-PCR using 18S rRNA as an internal control (Ambion, Austin, TX). Primers for actin were 5′-GCCATCCTCGTCTGGAC-CCTGGCT-3′ (forward) and 5′-GTGATGACCTGCGTCAG-GCAGC-3′ (reverse) (19). PCR conditions for actin were as follows: 25 cycles at 94°C for 30 s, 60°C for 60 s, and 72°C for 30 s. The size of the PCR product was 227 bp (19). Primers for α1-tubulin were 5′-CCATCAAGACAAAGCCAGCAT-3′ (forward) and 5′-CTCATAGGAGTCGATGCCCACCT-3′ (reverse) for α2-tubulin were 5′-ACAGGCGTTACCATGGAGACAGT-3′ (forward) and 5′-CCAGAGAGTGGTCTAGCGGAA-3′ (reverse). The size of the PCR products for α1- and β-tubulins was 300 bp. The PCR conditions were as follows: 30 cycles of 94°C for 30 s, 65°C for 60 s, and 72°C for 30 s. These two pairs of tubulin primers were designed based on the sequences of BT006731 and BC063610 (Genebank access number) using a web-based program, Genefisher (http://bibiserv.techfak.uni-bielefeld.de/genefisher/). The PCR products were separated on 2% agarose gels, and the pixel intensity of the bands was quantitated in Photoshop. The relative expression levels of target genes were normalized to 18S rRNA.

**Fabrication and mechanical loading of three-dimensional bioartificial tendon cultures.** The three-dimensional (3D) bioartificial tendon (BAT) cultures were fabricated in Tissue Train culture plates (Flexcell International, Hillsborough, NC), as described before but at lower 1:400 dilution in PBS). The cells were then rinsed with PBS twice and mounted on glass slides. Microtubules were stained with anti-α-tubulin and anti-β-tubulin monoclonal antibodies, respectively (Sigma). The permeabilized HTIFs were blocked with PBS containing 5% BSA and 2% goat serum at room temperature for 2 h and washed with PBS twice. Then the cells were incubated with the first antibody (1:1,000 for α-tubulin, 1:200 for β-tubulin, diluted in PBS) at 4°C overnight. After washing with PBS, the cells were incubated with Alexa Fluor 488-conjugated (α-tubulin) or Alexa Fluor 568 (β-tubulin) goat anti-mouse IgG (Molecular Probes; 1:200 diluted in PBS) at room temperature for 2 h. The cells were washed with PBS and mounted on glass slides. Cells were imaged using a LeicaSP2 AOB laser scanning confocal microscope (Leica Microsystems, Exton, PA) with a ×40 oil immersion objective.
density (50,000 cells/BAT rather than 250,000 cells/BAT) (12). This specialty culture plate allows for the molding of a 3D linear cell-populated matrix gel that is 30 × 4 × 4 mm. In brief, HTIFs were trypsinized, and the cell number was determined using a Coulter particle counter (Beckman Coulter, Hialeah, FL). Cells were mixed with type I collagen (Vitrogen, 2.1 mg/ml; Cohesion, Palo Alto, CA) at 500,000 cells/ml. One hundred microliters (100 μl) of cell-gel suspension were transferred to a space created by vacuum deformation of the rubber membrane into an underlying trough loader jig (30 × 4 × 4 mm space). After gelation, vacuum was released, culture fluid was added, and the cells were cultured in the 3D BAT matrix for 48 h in medium 199 containing 10% FBS. Cells were then brought to quiescence by reducing the serum concentration to 0.5% for 24 h. The cultures were subjected to uniaxial strain for 5 days at 3.5% elongation, 1 Hz, for 1 h per day with Flexcell’s Tension Plus cell strain system (model FX4000, Flexcell International, Hillsborough, NC) in the absence or presence of 100 pM IL-1β. After uniaxial loading, the cultures were fixed and stained with rhodamine-phalloidin, as described above. Images were recorded using an Olympus BX60 fluorescence microscope (OPELCO, Dulles, VA).

Determination of nonviable cells. At the termination of the experiments, HTIFs were released from the collagen gels using 1% type II collagenase (Worthington Biochemical, Lakewood, NJ). The number of nonviable cells was determined by a Trypan blue exclusion assay, according to the manufacturer’s protocol (Sigma).

Growth curves of human tenocytes. Human tenocytes were plated in 12-well plates and allowed to grow for 24 h in medium 199 ( Gibco) containing 10% FBS (HyClone, Logan, UT), 20 mM HEPES (pH 7.2, Gibco), and 1% penicillin/streptomycin solution (Gibco). Then serum concentration was reduced to 2%, and 100 pM recombinant human IL-1β were added. The media and IL-1β were refreshed daily. Cells were trypsinized, and cell numbers were counted on days 1, 3, and 5.

Statistics. All experiments were repeated at least three times for each patient. An unpaired Student’s t-test was used to test

Fig. 3. Effects of IL-1β treatment on the cytoskeleton of HTIFs. The representative results were shown here using the tenocytes from patient 1. Top: rhodamine-phalloidin staining of actin filaments. On day 1, the fluorescence intensity of actin filaments was reduced dramatically by IL-1β. On days 3 and 5, numerous short actin filaments were found in IL-1β-treated cells (thin arrow). Also, some thicker, but punctate, actin fibers were formed in ~25% of cells, but were not distributed evenly (thick arrow). Middle: immunostaining of α-tubulin. Microtubules were stained with anti-α-tubulin monoclonal antibody and visualized with Alexa Fluor 488-conjugated goat anti-mouse IgG. No obvious change in the structure of microtubules was observed. However, increased staining of microtubules was found from day 1. Bottom: immunostaining of β-tubulin. Microtubules were stained with anti-β-tubulin monoclonal antibody and visualized with Alexa Fluor 568-conjugated goat anti-mouse IgG. No obvious change in the structure of microtubules or staining intensity was observed. Scale bar is 50 μm.
for significant differences between groups. Changes at a level of \( P < 0.05 \) were considered significant. The values were expressed as means (SD).

**RESULTS**

*Measurement of Young’s modulus.* IL-1β treatment changed the cell shape dramatically. Most of the cells changed to a stellate shape with multiple processes extending from the central body (Fig. 1). The mean diameters of collagenase-detached, rounded HTIFs (patient 1) from control and IL-1β-treated groups were 17.5 (SD 1.96) and 18.6 μm (SD 2.09), respectively, and were not significantly different (\( P = 0.21, t\)-test). Similar results were found in HTIFs from patients 2 and 3. Using this method, previous studies have shown no dependence of the Young’s modulus on the cell diameter or on the ratio of cell diameter to micropipette diameter (25). The mean Young’s modulus was reduced by IL-1β in HTIFs from all three patients to 45% \([\text{patient } 1, \text{from } 439 (\text{SD } 235) \text{ to } 240 \text{ Pa (SD } 83.0)], \text{3} \% \text{[patient } 2, \text{from } 423 \text{ (SD } 299) \text{ to } 158 \text{ Pa (SD } 70.5)], \text{27} \% \text{[patient } 3, \text{from } 87 \text{ (SD } 15) \text{ to } 64 \text{ Pa (SD } 13)] \) of the control, nontreated levels (Fig. 2).

*Effects of IL-1β on the cytoskeleton of HTIFs.* Results of rhodamine-phalloloidin staining showed that the level of actin filaments in IL-1β-treated cells was dramatically reduced from day 1 compared with that in control cells (Fig. 3, top). Numerous short-actin fibers were found in IL-1β-treated cells from day 3 (thin arrow in Fig. 3, top). On days 3 and 5, thicker, but punctate, stress fibers were formed in ~25% of cells, but were not distributed evenly in the cells (thick arrow).

In Fig. 3, middle and bottom, the structure of microtubules, stained with anti-α-tubulin and anti-β-tubulin monoclonal antibodies, was not changed by IL-1β. However, immunochimical staining for α-tubulin was increased dramatically in IL-1β-treated cells from day 1. No obvious changes were found in β-tubulin staining.

*Actin and tubulin expression levels in HTIFs with and without IL-1β.* Results in Fig. 3 showed that IL-1β changed the levels of actin stress fibers and microtubules. To assess the influence of IL-1β on the steady-state mRNA levels of actin and tubulins, the relative expression levels of actin and tubulins were determined using a semiquantitative RT-PCR method. Results showed that the steady-state mRNA level of actin was reduced by 55% (SD 5.7) on day 1 but recovered to 90% (SD 4.0) of control on day 5 (Fig. 4A). In contrast to actin, the expression level of α1-tubulin was upregulated by IL-1β (Fig. 4B). α1-Tubulin expression level was increased by 34 (SD 14), 27 (SD 3.8), and 93% (SD 19), respectively, on days 1, 3, and 5. The steady-state mRNA level of β2-tubulin was not changed by IL-1β on days 1 and 3 and was increased on day 5 (Fig. 4C).

*IL-1β increased the tolerance of tenocytes to mechanical loading in 3D cultures.* In the absence of IL-1β, the mechanical loading regimen used in this study resulted in cell rounding in most of the rhodamine-phalloloidin-stained human tenocytes (Fig. 5A). Results of Trypan blue exclusion experiments showed that >90% of stretched cells were nonviable (Fig. 5B). However, addition of IL-1β maintained cell viability during 5 days of mechanical loading. Cell shape and density in the load plus IL-1β-treated group were similar to that in the untreated control group, as assessed by rhodamine-phalloloidin staining of the F-actin cytoskeleton and Trypan blue exclusion assay (Fig. 5).

*The proliferation rate of human tenocytes was not increased in the presence of IL-1β.* To confirm that the recovery of cell viability under mechanical loading in the presence of IL-1β was not due to IL-1β-induced cell proliferation, the growth...
curves of human tenocytes were measured in the absence or presence of IL-1β (Fig. 6). To minimize the interference of serum on IL-1β/H9252, the concentration of serum was reduced to 2% (cells at 2% serum did not show obvious growth). Under the experimental conditions, cell numbers were not changed during the 5-day culture period in the IL-1β-treated groups. In the control groups, cell numbers were increased by ~40% on day 5 compared with day 1.

DISCUSSION

Mechanical loading plays critical roles in cell differentiation, proliferation, tissue development, skeletal maintenance, and recovery postsurgery (6–8, 12, 44). One way that cells sense mechanical forces is through the deformation of the cytoskeleton (22, 24). In this regard, the mechanical properties of the extracellular matrix relative to those of the cell can significantly affect the micromechanical environment. For example, a mismatch in the Young’s modulus of cells relative to the matrix can significantly amplify the cell strains under the same applied load (1, 2, 17). For this reason, it is possible that changes in the mechanical properties of the extracellular matrix (i.e., due to IL-1β stimulation of MMPs) may cause cells to adapt their mechanical properties by reorganizing the F-actin cytoskeleton to maintain a strain setpoint (2). In certain cells, it is believed that mechanical signal transduction is initiated by cell deformation (13, 22, 24). A certain level of tensile pre-stress will develop upon cell attachment to the matrix, which is determined by the stiffness of the matrix, connections between the cell and matrix (focal adhesion sites), cell-cell connections, and the stiffness (modulus) of cells (15, 27). Changes in any of these properties could alter the intrinsic tension in the cytoskeleton and therefore affect cell responses to mechanical loads.

It has been reported that IL-1β stimulates the expression of MMPs and reduces the stiffness of extracellular matrix due to the degradation of matrix macromolecules (3, 7, 30, 37). It is likely that the cell modulus may be reduced by IL-1β to match the environmental change. In this study, it was shown that IL-1β reduced the Young’s modulus for each patient’s cells, although there was a large variation in values among patients (~430 vs. ~90.0 Pa). This variation may be due to the differences in individual patients in age, gender, or disease but...
may also be due to intrinsic differences among tendons powered by different muscles (25, 34). Cells with a lower Young’s modulus are more elastic and therefore may be able to withstand larger deformations before damage or failure. This hypothesis is supported by the results of mechanical loading experiments of 3D cultures in the present study (Fig. 5). The recovery of cell viability was not due to increased cell proliferation rate (Fig. 6). Results of previous studies have shown that mechanical loads stimulated the expression and release of IL-1β in human tenocytes (36). Therefore, the secretion of IL-1β under mechanical loading may represent a mechanism that allows cells to alter their interactions with extracellular matrix under extreme environments by reduction of their modulus and induction of MMPs, which can then be activated to degrade matrix and release cell-matrix contacts (40).

The determinants of mechanical properties of cells are not fully understood; however, it is believed that the elastic modulus is mainly determined by the distribution and structure of the cytoskeleton (35). By using cytoskeleton-disrupting chemicals, it has been found that disrupting F-actin filaments by cytochalasin D decreased the cell modulus (35), whereas disrupting the microtubular network by nocodazole or colcemid increased the cell modulus (43). Similar results were also reported by Kolodney and Wysolmerski (29) and Brown et al. (9). It has also been reported that changing the ratio of α-tubulin to β-tubulin will affect the structure of microtubules (42). The normal ratio of α-tubulin to β-tubulin is 1: overexpression of β-tubulin will disassemble microtubules and induce apoptosis. However, increasing α-tubulin does not obviously change the structure of microtubules (42). These results are consistent with the findings in the present study: that IL-1β may reduce the cell modulus by decreasing/disrupting actin filaments and increasing microtubules. Increasing the ratio of α-tubulin over β-tubulin did not change the structure of microtubules in human tendon cells. In other studies, however, opposite results were reported on the effects of microtubules on cell modulus (33, 35). In these two studies, cytochalasin D (2–3 h postaddition), but not colchicines, reduced the cell modulus of chondrocytes and normal rat kidney fibroblasts. It was also reported that cytochalasin D did not change the elastic modulus of Strongylocentrotus purpuratus embryos (<60 min treatment), even though F-actin was severely disrupted (11). These results indicate that the effects of cytoskeleton-disrupting drugs on elastic modulus changes are time sensitive. A more detailed investigation of the time-dependent changes of cytoskeletal structure will be needed to understand the long-term relationship to the elastic modulus of the cell. Also, cytoskeleton-disrupting drugs may initiate signaling pathways in addition to simply disrupting the cytoskeleton (28). However, releasing the cell modulus to a more compliant rather than a stiffer phenotype likely spares a cell from damage due to excessive strain.

Currently, several cell survival pathways have been reported (28, 39, 41). However, the mechanism by which cells survive extreme mechanical loading conditions has not been addressed. IL-1β, as a potential cell survival factor, has been reported to protect blast cells from apoptosis in suspension culture (38). NF-κB pathways activated by proinflammatory factors, such as tumor necrosis factor and IL-1β, also play an important role in anti-apoptosis (31, 41). Therefore, we hypothesize that IL-1β may mediate cell survival under extreme mechanical conditions by activating NF-κB pathway(s), which, in turn, affect the reorganization of the cytoskeleton and reduce the Young’s modulus of the cell (4, 18).

For the first time, we report that the mean Young’s modulus of HTIFs is reduced by IL-1β. Modulation of the cell’s elastic modulus may be useful in mechanical conditioning of tissue engineered constructs and as a treatment postsurgery to increase cell survival.

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DISCLOSURES

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