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IL-1β Sensitizes Intervertebral Disc Annulus Cells to Fluid-Induced Shear Stress

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Abstract Chronic inflammation and altered mechanical loading are implicated as contributors to intervertebral disc degeneration. Biomechanical and biochemical factors play a role in disc degeneration but have received limited study. Mechanically, intervertebral discs are sheared during bending or twisting of the trunk. Biochemically, IL-1β, detected in degenerative discs, promotes metalloproteinase expression. We hypothesized that disc cells might respond to shear stress and IL-1β in a calcium signaling response. We measured the effect of single and combined stimuli on intracellular calcium concentration ($[Ca^{2+}]_{ic}$) and signaling. Cells were isolated from annulus tissue, cultured to quiescence, plated on collagen-bonded Culture Slips® and incubated with Fura-2AM. Cells then were incubated in IL-1β. Cell response to the effects of fluid flow was tested using FlexFlo™, a laminar flow device. Human annulus (hAN) cells responded to laminar fluid flow with a one to three-fold increase in $[Ca^{2+}]_{ic}$. IL-1β alone produced a small, transient stimulation. hAN cells pretreated with IL-1β responded to shear with a more dramatic and sustained increase in $[Ca^{2+}]_{ic}$, six to ten-fold over basal level, when compared to shear then IL-1β or shear and IL-1β alone ($P < 0.001$ for all comparisons). This is the first study documenting synergism of a signaling response to biomechanical and biochemical stimuli in human disc cells. IL-1β treatment appeared to ''sensitize'' annulus cells to mechanical load. This increased responsiveness to mechanical load in the face of inflammatory cytokines may imply that the sensitivity of annulus cells to shear increases during inflammation and may affect initiation and progression of disc degeneration. J. Cell. Biochem. 82: 290–298, 2001.

Key words: fluid flow; interleukin-1-beta; intervertebral disc

Degeneration of the intervertebral disc and altered mechanical loading are implicated as contributors to the development of adult spinal deformity and as a major cause of low back pain [Benner and Ehni, 1979; Grubb et al., 1988; Kornberg, 1989; Holm, 1993; O'Brien et al., 1993; Moneta et al., 1994]. While the factors that initiate and perpetuate disc degeneration are not well understood, both biomechanical and biochemical factors play a role [Holm and Nachemson, 1983; Holm, 1993; Buckwalter, 1995; Handa et al., 1997; Hashizume et al., 1997; Kang et al., 1997].

The function of the intervertebral disc is to provide the spine with mobility while retaining axial stability. The spine is structured to constrain motion at high loads and to allow flexibility at low loads [Simon et al., 1994]. The specialized structures of the disc, the outer and inner layers of the tough annulus fibrosus, and the central gelatinous nucleus pulposus, confer on the spine the ability to resist substantial mechanical stresses. The annulus fibrosus is 60–70% water, whereas the nucleus pulposus is 90% water [Sedowofia et al., 1982; Simon et al., 1994]. The two components (inner and outer regions) of the annulus fibrosus comprise interwoven lamellae of primarily collagen types I and II, which are commonly concentrated in tensile and compression load bearing tissues respec-

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tively. The nucleus pulposus contains randomly oriented collagen II fibers, which are commonly present in tissue exposed to compressive loads [Sedowofia et al., 1982; Simon et al., 1994; Nerlich et al., 1997]. Maintenance of these disc tissues and the molecular interactions of the extracellular matrix (ECM) are necessary for adequate biomechanical integrity and function of the intervertebral disc. Any disturbance in the balance between these tissues invariably leads to alteration in composition, functional impairment, and degeneration.

The disc is under some load at all times, even at rest, due to the forces of the muscles, ligaments and hydrostatic pressures within the disc [Nachemson, 1966]. During movement of the spine the disc is exposed to compression, tension and shear forces. Physiologic loading is beneficial to maintain metabolism and function of the disc [Holm and Nachemson, 1983; Oshima et al., 1995; Ishihara et al., 1996]. Mechanical loading, such as shear stress, compression, tension, and vibration of intervertebral discs contributes to the disc's metabolism, especially proteoglycan and protein synthesis [Handa et al., 1997]. Research indicates that hydrostatic pressure directly affects disc metabolism by maintaining synthesis and degradation of the matrix [Handa et al., 1997]. Intradiscal hydrostatic pressure increases and decreases with load, thus fluid efflux or influx from the nucleus pulposus to the annulus fibrosus increases, respectively [Simon et al., 1994; Handa et al., 1997]. As a result, intervertebral discs are constantly subjected to fluid-induced shear stresses from the fluid movement within the disc. Mechanical loading can also stimulate disc changes through direct cellular effects, as it appears to in cartilage, or indirectly through alterations in the local environment — blood flow, hydration, nutrient diffusion, etc. Cultured human annulus (hAN) cells mechanically stimulated by indentation with a micropipette or by fluid-induced shear stress responded with a dramatic increase in intracellular calcium. Connective tissue cells from bone, cartilage, ligament and tendon, and endothelial cells also respond to fluid-induced shear stress by increasing intracellular calcium concentration $[Ca^{2+}]_{ic}$ or nitric oxide (NO) [Hung et al., 1996, 1997; Das et al., 1997; Yellowley et al., 1997; Francke et al., 2000; Malek et al., 1999; Yellowley et al., 1999; Elfervig et al., 2000]. Fluid-induced shear stress increased mRNA expression of transforming growth factor-β1 (TGF-β1) in human osteoblast-like cells [Sakai et al., 1998]. Moreover, rabbit tendon paratenon cells, which are involved in tendonitis, respond to fluid-induced shear stress by increasing gene expression for matrix metalloproteinase 3. Thus, mechanical loading can induce signaling pathways that can result in specific gene expression.

While physiologic loading helps to maintain metabolism and function in intervertebral discs, excessive mechanical loading appears to be detrimental both experimentally and clinically. Epidemiological and cohort studies have shown an association between excessive loading of the spine and increased problems with low back pain, disc degeneration, and disc herniation [Frymoyer et al., 1983; Damkot et al., 1984; Hulshof and van Zanten, 1987; Pope et al., 1987; Hansson and Holm, 1991; Bovenzi and Zadini, 1992; Miyashita et al., 1992; Pope and Hansson, 1992; Seidel, 1993; Wilder, 1993; Bovenzi, 1996]. Degenerative discs contain increased levels of matrix metalloproteinases (MMPs) and inflammatory mediators [Kang et al., 1996; Nemoto et al., 1997]. Asymmetric loading across the disc may also further stimulate the production of endogenous cytokine mediators, which in turn increases the response to the mechanical stimulus. Crean et al. [1997] has demonstrated in scoliotic discs that the expression of MMPs differed between the concave and convex sides of the disc. Furthermore, cytokines, particularly interleukin-1 (IL-1), and growth factors can potentiate metabolic activity in disc tissue [Shinmei et al., 1988]. IL-1 stimulates several different signal transduction pathways including G-protein dependent and kinase pathways that lead to increased levels of chemical mediators such as cyclic AMP, interleukin-2 (IL-2), and prostaglandin E_2 (PGE_2) [Bankers-Fulbright et al., 1996]. IL-1 also promotes gene expression of proinflammatory proteins such as cyclooxygenases and other interleukins [Bankers-Fulbright et al., 1996]. IL-1β effects disc cell metabolism by increasing expression of MMPs, NO, PGE_2, and interleukin-6 (IL-6) [Kang et al., 1997]. hAN cells express the IL-1β receptor and consequently show an increase in $[Ca^{2+}]_{ic}$ in response to a bolus of IL-1β [Minchew et al., 2000]. Other connective tissue cells, such as cells from tendon, respond in a similar fashion when exposed to IL-1β [Guyton et al., 2000].

The process of disc degeneration likely results from both biochemical and mechanical stimulation of cells, activating a matrix destructive pathway. However, the interaction between biomechanical and biochemical stimulation of intervertebral disc cells has not yet been documented. We hypothesized that fluid-induced shear stress and IL-1β treatment would evoke a synergistic response in hAN cells. In order to evaluate this interaction, we measured the effect of the single and combined stimuli on an immediate cell response, changes in $[Ca^{2+}]_{ic}$.

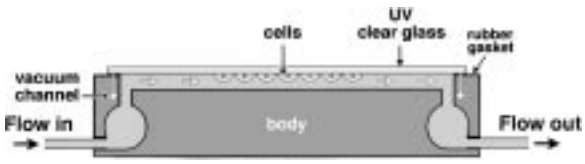

Fig. 1. Schematic of the FlexFlo parallel-plate flow chamber, which provides laminar fluid flow in the viewing region of the chamber. The chamber has inlet and outlet valves through which the perfusate enters and leaves the chamber, respectively. The glass slide is placed cells down on top of the chamber and held in place by a vacuum. The rubber gasket provides a seal that prevents leaking.

MATERIALS AND METHODS

Cell Isolation

hAN tissue from the lumbar region of the spine was obtained from the uninjured regions of the discarded intervertebral disc specimens of five female patients, ranging in age from 12 to 51 years with an average age of 26.4 years, undergoing surgery for anterior scoliosis release, disc herniation, or traumatic injury. These specimens are arbitrarily designated hAN-1, -2, -3, -4, and -5. The annulus fibrosus was dissected from the nucleus pulposus, washed twice in Medium 199, transferred to a roller tube and digested with 0.5% bacterial collagenase in Medium 199 with Hepes buffer, pH 7.2 for 45 min at 37°C in a CO_2 incubator. Annulus cells were plated at 25k cells/cm^2 in Medium 199 with 10% fetal calf serum, 0.5 mM ascorbate, 20 mM Hepes pH 7.2, and antibiotics. Cells were passaged using 0.05% trypsin in Ca^{2+} free PBS at pH 7.2. hAN cells were used at passage 3–15. Under the in vitro conditions described above, hAN cells express aggrecan and collagen type I but not type II indicating that their phenotype is maintained in culture [Minchew et al., 1999].

Calcium Measurements

In order to measure the $[Ca^{2+}]_{ic}$ in response to cytokines or fluid flow, cells were plated in complete medium as described above on Culture Slip® growth surfaces ($80 \times 35 \times 0.5$ mm collagen-bonded, low UV-absorbing, glass rectangles). The Culture Slips® also comprise the top view port of the parallel plate, fluid flow, shear device (FlexFlo™ chamber, Flexcell Intl. Corp., McKeesport, PA) (Fig. 1). In earlier experiments, cells were plated at 25k cells/cm^2. In later experiments, cells were plated in micromass spot cultures at 3k cells/10 μl spot ($n = 2$ spots/slide/patient) in the central region of a Culture Slip®. Spot culturing prevented the response to fluid flow from being caused by the release of mediators from upstream cells and allowed us to measure the response in cells at the front of the flow field. Cells were grown to quiescence by halving the medium on Days 3 and 5 with serum-free Medium 199. On Days 6–10 after plating, hAN cells were removed from culture, rinsed in Earles' Balanced Salt Solution (EBSS) with calcium and magnesium, and incubated in the dark at room temperature in 5 μM Fura-2AM in EBSS with a final concentration of 0.1% Pluronic-127 and 0.5% DMSO for 60 min. After incubation, the cells were rinsed in EBSS to remove any unincorporated Fura-2AM. The Culture Slips® were then transferred to the FlexFlo™ apparatus (cells facing the flow stream), which was mounted on the stage of an upright fluorescence microscope equipped with a xenon light source, mechanical filter wheel (Olympus, Metaltek, Raleigh, NC) and a CCD camera with a light intensifier. Image I analysis software (Universal Imaging, West Chester, PA) was used to quantitate the $[Ca^{2+}]_{ic}$. A ratio-dye method, with 340/380 nm excitation and 510 nm and above emission, was used to convert the fluorescence intensity of the labeled hAN cells to $[Ca^{2+}]_{ic}$ based on known calcium standards. 20–30 cells/field were outlined and the pixel intensity quantified for each cell. A baseline calcium value was determined for each cell as well as a value after excitation with IL-1β or shear stress.

Biomechanical and Biochemical Stimuli

The responses to fluid flow of hAN cells from all specimens (hAN 1, 2, 3, 4, and 5) were tested at shear stress levels of 1, 3, 5, 10, 15, 20, and 25 dynes/cm^2 using a FlexFlo™ parallel-plate flow

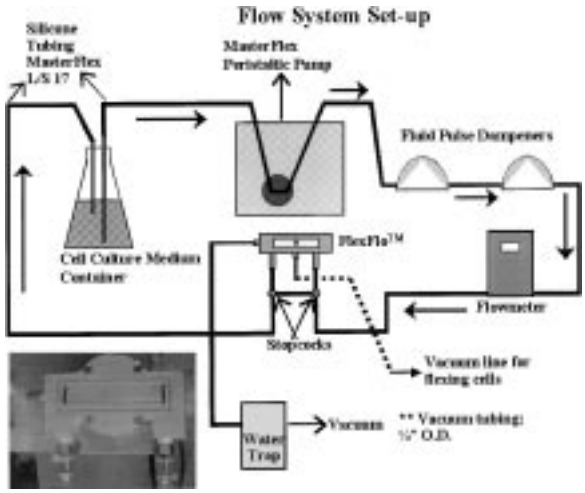

Fig. 2. Schematic of the flow system set-up. The system is a closed system in which the perfusate is recycled back to the same container. The MasterFlex Pump is a roller pump that pumps the perfusate through the MasterFlex silicone tubing (size 17). The pulse dampeners remove the air in the lines. The flowmeter indicates the flow rate of the perfusate through the system. The FlexFlo™ chamber is a parallel plate laminar flow device (see Fig. 1). The stopcocks allow the user to alter the flow away from the chamber when changing out the slide and when removing the initial air bubbles from the tubing. The system has two vacuum lines. One holds the slide with the cells over the flow chamber and the second provides a vacuum that can ''flex'' the cells.

device and a MasterFlex™ peristaltic pump, equipped with silicone tubing, two pulse dampeners, and a flowmeter (Fig. 2). Cells were repeatedly stimulated by fluid flow with a 15 min incubation at rest at room temperature following each stimulation. To determine the source of calcium in the signaling response, the cells from two of the specimens (hAN-3, -4) were subjected to a perfusate with calcium (EBSS with 1.8 mM Ca^{2+}, 0.8 mM Mg^{2+}, and 20 mM Hepes pH 7.2) and without calcium (EBSS with 20 mM Hepes pH 7.2). hAN cells from three of the specimens (hAN-1, -4, -5) were tested for their response to IL-1β at concentrations of 50 and 500 pM and 1 and 5 nM. This response also was measured in the presence and absence of extracellular Ca^{2+} in the medium. In order to determine the combined effect of IL-1β and fluid-induced shear stress, hAN cells from all specimens were incubated for 30 min in 1 nM IL-1β, rinsed with EBSS and subjected to fluid flow at 1, 5, 10, 15, and 20 dynes/cm^2. hAN cells from two of the specimens (hAN-2, -4) were pretreated with 1nM IL-1β and then subjected to a perfusate without calcium, as described above. hAN cells from three of the specimens (hAN -1, -4, -5) were also subjected to fluid flow at varying shear stress levels then treated with 1nM IL-1β. For all testing conditions involving fluid-induced shear stress, the flow paradigm was steady laminar flow with fluid flow being applied for 1–3 min. Furthermore, as a control, no flow or baseline $[Ca^{2+}]_{ic}$ measurements were collected for an average of 60 sec prior to flow.

Statistical Analysis

All data were entered into Microsoft Excel spreadsheets and analyzed using SigmaStat (SPSS Science, Chicago, IL), a statistical software program. Levels of statistical significance ($P < 0.05$) were determined with a One Way ANOVA and a Tukey post-hoc test to determine significance between groups. The highest and lowest increase in $[Ca^{2+}]_{ic}$ was thrown-out for all conditions. The basal level was determined by averaging the $[Ca^{2+}]_{ic}$ of the no flow condition over 60 sec. A cellular response was mathematically represented as a cell whose average $[Ca^{2+}]_{ic}$ response increased two standard deviations over its basal level. The mean peak $[Ca^{2+}]_{ic}$ is the average over all responding cells of the maximum $[Ca^{2+}]_{ic}$ increase per cell for one trial.

RESULTS

hAN cells responded to the effects of fluid flow at shear stress levels of 1, 3, 5, 10, 15, 20, and 25 dynes/cm^2 with a gentle, transient rise in $[Ca^{2+}]_{ic}$ that was 50 to 300% above baseline values. The response began ~30 sec after the initiation of flow and continued for another 30–60 sec. Data in Figure 3 represent a typical dose response plot for responding hAN cells subjected to varying magnitudes of fluid-induced shear stress. IL-1β alone stimulated cells modestly and transiently as seen by the increase in $[Ca^{2+}]_{ic}$ that was 50–250% above baseline values (Fig. 4). The response to the addition of IL-1β was immediate and lasted 30 to 60 sec. hAN cells subjected to fluid flow at varying shear stress levels then treated with IL-1β within 15 min after flow was stopped, increased $[Ca^{2+}]_{ic}$, but the response was not synergistic (Fig. 4). $[Ca^{2+}]_{ic}$ increased 50–200% above basal values. Twelve to fifty percent of the hAN cells responded to the above stimuli with an increase in $[Ca^{2+}]_{ic}$ two standard deviations over baseline values (Fig. 4). In addition, for all treatments, the cells could be restimulated within 15 min after returning to basal level (data not

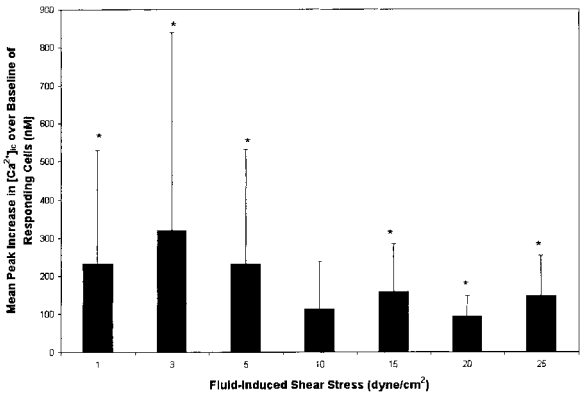

Fig. 3. Dose response of hAN cells to fluid-induced shear stress. The data in this graph represent the average increase (mean ± SD) in the peak $[Ca^{2+}]_{ic}$ where n = 34, 39, 75, 84, 64, 53, and 43 responding cells for the 1, 3, 5, 10, 15, 20, and 25 dyne/cm^2 shear stresses, respectively. All shear stress levels induced a significant (*$P < 0.05$) increase in$[Ca^{2+}]_{ic}$ over basal levels except 10 dynes/cm^2.

shown). Only 1% of the cells spontaneously responded in the absence of shear stress.

hAN cells pretreated for 30 min with 1 nM IL-1β responded to fluid flow with a more dramatic increase in $[Ca^{2+}]_{ic}$. Data in Figure 4 illustrate this increase in $[Ca^{2+}]_{ic}$ as compared to the other stimuli (shear alone, IL-1β alone, and shear then IL-1β treatment). Figure 5 shows a typical increase in $[Ca^{2+}]_{ic}$ for cells pre-treated with IL-1β and then sheared at varying flow rates as compared to those cells that were subjected to a shear stress alone. Sixty percent of the IL-1β pretreated cells responded to fluid flow with a

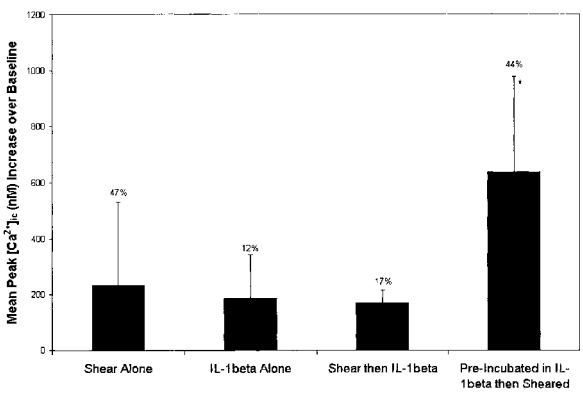

Fig. 4. A compendium of the Ca^{2+} responses to the different mechanical stimuli: shear stress alone at 5 dynes/cm^2 (n = 64), 1nM IL-1β alone (n = 5), shear stress then 1nM IL-1β (n = 14), and pre-incubation in 1nM IL-1β for 30 min then shear stress at 5 dynes/cm^2 (n = 100). Above each column is the percent cells responding. Pre-incubation with 1nM IL-1β then shear of 5 dynes/cm^2 was statistically significant (*$P < 0.05$) from the other stimuli. "n" is the number of responding cells.

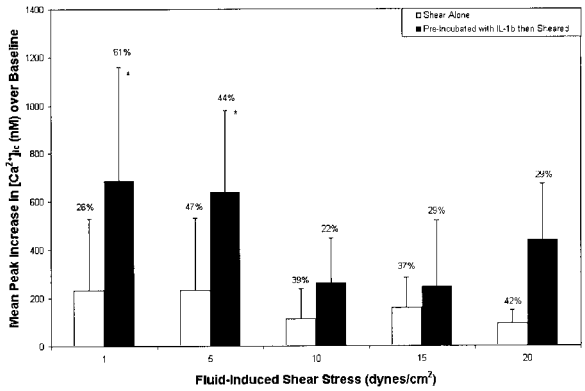

Fig. 5. Pre-incubation with 1nM IL-1β increases the sensitivity of the response of hAN cells to the effects of fluid flow. The increase in $[Ca^{2+}]_{ic}$ over baseline was statistically significant ($P < 0.05$) for cells pre-treated with IL-1β then sheared at 1 and 5 dynes/cm^2 as compared to those cells that were subjected to fluid- shear stress alone. Above each column is the percent cells responding. For the shear alone condition, n = 34, 75, 84, 64, 73 responding cells for the shear stress levels of 1, 5, 10, 15, and 20 dynes/cm^2, respectively. For the pre-incubation with IL-1β then shear condition, n = 41, 100, 8, 49, 17 responding cells for the shear stress levels of 1, 5, 10, 15, and 20 dynes/cm^2, respectively. The * indicates statistical significance of ($P < 0.05$).

robust and sustained increase in intracellular calcium levels that was 100–800% greater than basal values at the low flow conditions. However, at higher shear stress levels only 20–30% of the cells IL-1β pretreated responded. In contrast, the cells subjected to shear alone had a higher percentage of cells responding at higher rather than lower shear stress levels (Fig. 5). The $[Ca^{2+}]_{ic}$ remained elevated for up to 30 min post-flow in one group of cells pre-treated with IL-1β and then subjected to shear stress as depicted in Figure 6. Within 15 min after the $[Ca^{2+}]_{ic}$ returned to baseline values, hAN cells could be restimulated by fluid flow to increase $[Ca^{2+}]_{ic}$ six- to ten-fold above baseline (data not shown). An experiment was performed on cells pretreated with IL-1β then subjected to fluid shear at 15 dynes/cm^2 in Ca^{2+}-free medium to test if the prolonged increase in $[Ca^{2+}]_{ic}$ was an artifact due to drift in the baseline. The results indicated that after 25 min post-flow, there was no significant increase in $[Ca^{2+}]_{ic}$ over baseline (data not shown). Thus, the prolonged response was not an artifact due to drift.

In order to determine the source of calcium in the signaling response, hAN cells were subjected to both stimuli in the absence of extracellular calcium. hAN cells did not significantly respond to any of the stimuli by increasing $[Ca^{2+}]_{ic}$ in the absence of extracellular calcium

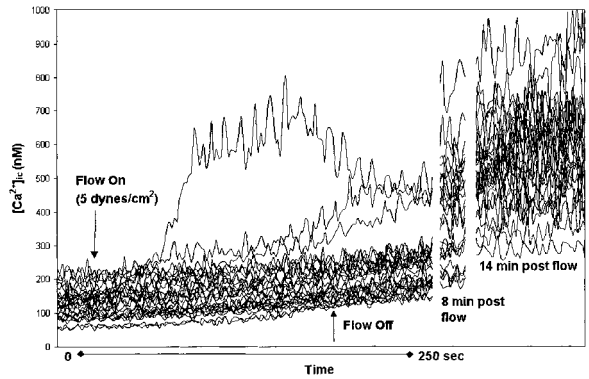

Fig. 6. The increase in $[Ca^{2+}]_{ic}$ was observed up to 30 min post-stimulation at 5 dynes/cm^2 shear stress in one group of cells. Each line (n = 26) represents the response of an individual cell. Three regions of the response are represented here: (1) the first 250 sec during the flow stimulation, (2) 8 min after the flow stimulation, and (3) 14 min after the flow stimulation. A similar response was observed at 15 dynes/cm^2 shear stress in the same group of cells.

(Fig. 7), indicating a requirement for exogenous calcium.

DISCUSSION

This study is the first to document a synergistic signaling response to a cytokine and shear stress stimulation in intervertebral disc cells. The effects of IL-1β pre-treatment on the $[Ca^{2+}]_{ic}$ response of annulus cells to fluid flow were clearly synergistic. hAN cells responded at a lower level of shear stress with a marked increase in intracellular calcium level that was 6–10 fold above basal levels. This high level was sustained for up to 30 min in one group of cells, much longer than was observed with shear

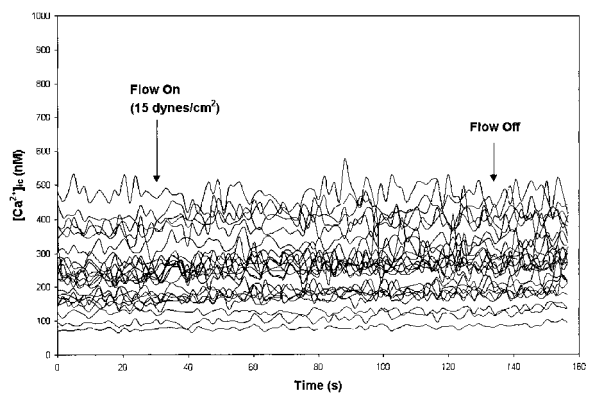

Fig. 7. hAN cells pretreated with 1 nM IL-1β did not respond to 15 dynes/cm^2 shear stress in the absence of extracellular calcium. The same response was seen in hAN cells that were that were only sheared or only treated with IL-1β.

stress without IL-1β pre-treatment or with IL-1β alone. In addition, more cells were initially recruited in the response at lower shear stress levels. IL-1β pre-treatment "sensitized" annulus cells to mechanical load, since applying a fluid-induced shear stress to the cells prior to adding the IL-1β did not have the same affect.

Interactions between biomechanical and biochemical stimulation have been noted in other connective tissues. Banes et al. [1995b] showed that avian flexor tendon cells responded mitogenically to substrate strain and platelet-derived growth factor (PDGF-BB) in a synergistic manner. Osteoblast-like cells (ROS 17/2.8) responded to cyclic substrate tension by mineralizing nodules in vitro, a response that was blocked by the L-type calcium channel blocker, Verapamil [Vadiakas and Banes, 1992]. A link between biomechanical and biochemical signaling in the regulation of bone was suggested in a study on mechanical loading and gene expression in human osteoblast-like cells [Sakai et al., 1998]. Fluid shear stress increased the mRNA levels of TGF-β1 in the osteoblast-like cells; however, Verapamil and gadolinium, an inhibitor of stretch-activated Ca^{2+} channels, blocked this response. These results indicated the involvement of calcium channels in the signaling pathway and a connection between biomechanical and biochemical signals in the regulation of bone formation [Sakai et al., 1998]. Previous work with annulus cells has demonstrated an interaction between extracellular messengers and mechanical stimulation. ATP treatment and mechanical stimulation of hAN cells both caused a significant increase in intracellular calcium. Mechanical stimulation of a single cell also resulted in cell-cell communication of the stimulus by way of an intercellular calcium wave. However, pretreatment of annulus cells with ATP transiently blocked their responsiveness to a direct mechanical stimulus and inhibited cell–cell signaling [Minchew et al., 1999]. Cellular responses to mechanical and biochemical stimuli may act through both common and independent pathways that are both positively and negatively regulated. The diversity of pathways for mechano-responsiveness belies a vital redundancy in systems for stimulus detection [Banes et al., 1995a].

In the absence of extracellular calcium, hAN cells did not significantly respond to fluid flow or treatment with IL-1β by increasing $[Ca^{2+}]_{ic}$.

These data illustrate that both the signaling pathway involved in the affect of fluid flow on the increase in $[Ca^{2+}]_{ic}$ and the IL-1β receptor signaling pathway are dependent on extracellular calcium. This observation indicates the linkage of a common pathway involving a calcium channel. A similar calcium-dependent response has been reported in human tendon surface cells and bovine articular chondrocytes subjected to fluid flow with a calcium-free perfusate [Yellowley et al., 1997; Francke et al., 2000].

The long term effects and clinical relevance of the sensitization of annulus cells to IL-1β remain unknown, since the downstream effects of the sustained calcium signal have yet to be evaluated. However, inflammatory cytokines such as IL-1β stimulate production of metalloproteinases that degrade matrix, including proteoglycans, and thus likely contribute to disc degeneration and diminished function [Bankers-Fulbright et al., 1996]. Data indicate that loss of proteoglycans is one of the main biochemical changes in the annulus of degenerative discs [Stevens et al., 1982]. In addition, this study demonstrates that IL-1β appears to have direct cellular effects that increase the susceptibility or response of cells to mechanical loading. If indeed, as it is postulated, the presence of IL-1β renders annulus cells more susceptible to injury from excessive load, this would have important clinical implications. This could be an important factor in annulus failure in degenerative discs resulting in tears and herniations. Additionally, this interaction could play a role in the progression, if not the etiology of deformity.

Alternatively, the response to IL-1β and shear stress may be protective to the intervertebral disc, serving to close calcium channels to prevent further response to persistent or additional shear stress. However this potential blunted response has not been investigated. Furthermore, higher flow rates may elute IL-1β from the its receptors, and thus blunt the Ca^{2+} response. Accordingly, future studies will attempt to further elucidate the interactions of biochemical and biomechanical stimuli in the response cascade. Our results, however, suggest that inflammatory cytokines increase the sensitivity of intervertebral discs to mechanical load, which could result in the release of matrix degenerative mediators and lead to further disc degeneration. It is unclear in disc degeneration as to which comes first, a biochemical change within the disc or abnormal loading. However, it appears that the two conditions are interrelated and that once the process is initiated, inflammatory mediators may increase mechanical responsiveness.

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