Integrin-regulated Secretion of Interleukin 4: A Novel Pathway of Mechanotransduction in Human Articular Chondrocytes

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Abstract. Chondrocyte function is regulated partly by mechanical stimulation. Optimal mechanical stimulation maintains articular cartilage integrity, whereas abnormal mechanical stimulation results in development and progression of osteoarthritis (OA). The responses of signal transduction pathways in human articular chondrocytes (HAC) to mechanical stimuli remain unclear. Previous work has shown the involvement of integrins and integrin-associated signaling pathways in activation of plasma membrane apamin-sensitive Ca^{2+}-activated K^+ channels that results in membrane hyperpolarization of HAC after 0.33 Hz cyclical mechanical stimulation. To further investigate mechanotransduction pathways in HAC and show that the hyperpolarization response to mechanical stimulation is a result of an integrin-dependent release of a transferable secreted factor, we used this response. Neutralizing antibodies to interleukin 4 (IL-4) and IL-4 receptor α inhibit mechanically induced membrane hyperpolarization and anti–IL-4 antibodies neutralize the hyperpolarizing activity of medium from mechanically stimulated cells. Antibodies to interleukin 1β (IL-1β) and cytokine receptors, interleukin 1 receptor type I and the common γ chain/CD132 (γ) have no effect on mechanically induced membrane hyperpolarization. Chondrocytes from IL-4 knockout mice fail to show a membrane hyperpolarization response to cyclical mechanical stimulation. Mechanically induced release of the chondroprotective cytokine IL-4 from HAC with subsequent autocrine/paracrine activity is likely to be an important regulatory pathway in the maintenance of articular cartilage structure and function. Finally, dysfunction of this pathway may be implicated in OA.

Key words: chondrocyte • mechanotransduction • integrin • interleukin 4 • ion channels

ARTICULAR cartilage covers the ends of long bones within synovial joints and protects the underlying bone against shearing and compressive forces. Cartilage is composed of a proteoglycan and collagen-rich extracellular matrix containing chondrocytes. Collagen forms a meshwork that imparts tensile strength, and proteoglycans form large aggregates that provide resistance to compression (Stockwell, 1991). The maintenance of cartilage matrix integrity is critically dependent on mechanical stimulation and cartilage thickness reflects the total load transmitted by the joint. Experiments on whole animals with intact joints have shown that abnormal loading, whether increased or decreased, influences cell metabolism and results in cellular and biochemical changes that lead to cartilage breakdown and the development of osteoarthritis (OA[1]; Mow et al., 1992). In vitro experiments with chondrocytes in culture have demonstrated a variety of physiological and biochemical responses to cyclical mechanical stimuli. These include changes in membrane potential, intracellular calcium concentration, and cAMP levels, and inhibition or stimulation of glycosaminoglycan production (Veldhuijzen et al., 1979; Urban, 1994).

Cyclical and static mechanical stimulation are well recognized as having a variety of effects on a number of different cell types, including bone cells, chondrocytes, vascular

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1. *Abbreviations used in this paper:* CM, conditioned medium; γc, common gamma chain; ECM, extracellular matrix; HAC, human articular chondrocytes; IFN-γ, interferon gamma; IL, interleukin; IP3, inositol triphosphate; MMP, matrix metalloproteinase; OA, osteoarthritis; PIS, pressure-induced strain; PLC, phospholipase C; PKC, protein kinase C; RT-PCR, reverse transcriptase-PCR; SK, small conductance Ca^{2+}-dependent K^+ channels; TGF-β1, transforming growth factor β1; TIMP, tissue inhibitor of matrix metalloproteinase.

endothelium, and smooth muscle, from tissues normally exposed to mechanical forces. Mechanical signals imparted by stretch, pressure, tension, fluid flow, or shear stress rapidly lead to the activation of multiple intracellular signaling molecules and pathways, including opening of stretch activated and calcium selective ion channels (Sachs, 1988), protein tyrosine phosphorylation (Yano et al., 1996), inositol lipid metabolism (Prasad et al., 1993), and activation of protein kinase C (PKC; Kimono et al., 1996). Activation of these and other signaling pathways in turn leads to changes in gene expression and protein synthesis of important regulatory mechanisms controlling tissue structure and function, e.g., PDGF production by smooth muscle cells (Wilson et al., 1993); nitric oxide and prostaglandin production by endothelial cells (Davies, 1995); proteoglycan synthesis by chondrocytes (Veldhuijzen et al., 1979); and bone matrix synthesis by bone cells (Harter et al., 1995).

The routes by which a particular mechanical signal is transduced into an intracellular response are being defined and evidence for a role for integrins is increasing (Wang et al., 1993; Shyy and Chien, 1997). Integrins are a family of heterodimeric (α and β chain) transmembrane glycoproteins that form specific receptors for extracellular matrix (ECM) proteins (Hynes, 1992). Many of the signal transduction and gene expression events activated by mechanical stimuli are identical to those induced by integrin-mediated cell adhesion (Hynes, 1992; Shyy and Chien, 1997). Integrins associate with signaling molecules in the focal adhesion complex that acts both as a signaling device and a connection to the cytoskeleton through which they can influence gene expression and control cell growth and function. Experimental work has provided further evidence that integrins may act as mechanoreceptors in a variety of cell types. Integrins support a force-dependent stiffening response in endothelial cells (Wang et al., 1993) and are involved in shear stress–dependent vasodilatation of coronary arteries (Muller et al., 1997) and transmitter release from motor nerve terminals (Chen and Grinell, 1995). Also integrins were shown to be necessary for mechanically induced activation of ERK-2 and JNK-1 intracellular signaling pathways in cardiac fibroblasts (MacKenna et al., 1998) and the membrane hyperpolarization and depolarization responses of human articular chondrocytes (HAC) and bone cells to cyclical mechanical strain (Salter et al., 1997; Wright et al., 1997).

We have developed a technique for applying controlled forces to cultured cells allowing direct demonstration that mechanical signals can be transmitted across ECM–cell contacts (Wright et al., 1992, 1996, 1997). Using this technique we have stimulated mechanically sensitive cells including fibroblasts, human bone cells, and chondrocytes. As a result, several electrophysiological, biochemical, and molecular responses were affected, including changes in cell membrane potential, protein-tyrosine phosphorylation (paxillin and FAK125), and c-fos activation, and (in the case of chondrocytes) increased production of aggrecan mRNA and proteoglycan synthesis (Wright et al., 1992, 1996, 1997; Salter et al., 1997). We have used the mechanically induced changes in membrane potential to dissect in detail molecules involved and pathways activated as a result of cyclical mechanical stimulation. The electrophysiological response to mechanical stimulation occurs within 20 min, is dependent on the frequency of mechanical stimulation, and is also cell-type specific (Salter et al., 1997; Wright et al., 1997). Stimulation at 0.33 Hz (2 s on/1 s off) for 20 min at 37°C causes both human chondrocytes and bone cells to undergo membrane hyperpolarization because small conductance Ca^{2+}-dependent K^+ channels (SK) open. In contrast, stimulation at 0.104 Hz (2 s on/7.6 s off) for 20 min with the same degree of microstrain results in membrane depolarization because the tetrodotoxin-sensitive Na^+ channels are activated. Fibroblasts, on the other hand, undergo membrane depolarization at 0.33 Hz and hyperpolarization at 0.1 Hz (Wright et al., 1992).

In this model system, signaling via integrins and integrin-associated signaling molecules (including actin cytoskeleton and tyrosine protein kinases) is necessary for both the hyperpolarization and depolarization responses to mechanical stimulation (Wright et al., 1996, 1997; Salter et al., 1997). However, the 0.33-Hz hyperpolarization response is inhibited by antibodies to α5 integrin and β1 integrins, whereas the 0.104-Hz depolarization response is inhibited by antibodies to $\alpha V\beta 5$ and not by anti-α5 integrin antibodies. This suggests specific roles of particular integrins in the transduction of different forms of mechanical stimulation to cells (Salter et al., 1997). Furthermore, stretch sensitive ion channels, phospholipase C (PLC), the inositol triphosphate (IP3) Ca^{2+}-calmodulin pathway, and PKC appear to be involved in the production of the hyperpolarization response only.

Studies in osteoblasts and endothelial cells have demonstrated the production of soluble factors, such as prostaglandins and nitric oxide, in response to mechanical stimulation (Somjen et al., 1980; Ayajiki et al., 1996). The purpose of the study was to investigate whether soluble mediators, in particular cytokines including interleukin 1β (IL-1β), IL-4, and transforming growth factor β1 (TGF-β1) that are recognized as having important roles in regulation of chondrocyte function via autocrine and paracrine signaling, were involved in the membrane hyperpolarization response of HAC to mechanical stimulation.

Materials and Methods

Isolation of Chondrocytes

Postmortem articular cartilage was aseptically removed from macroscopically normal femoral condyles and tibial plateaux of human knee joints. Donors had died from a variety of diseases unrelated to the locomotor system and were undergoing routine hospital autopsy. Cartilage was sampled from 8 males (mean age, 68 yr; range 58–83 yr) and 17 females (mean age, 76 yr; range 37–93 yr). Cartilage from different anatomical regions of the knee joint were pooled and chondrocytes were isolated by sequential enzyme digestion at 37°C in 95% air/5% CO_2 with 0.25% trypsin (GIBCO BRL) for 30 min and 3 mg/ml collagenase (type H; Sigma Chemical Co.) for up to 24 h as described previously (Wright et al., 1996). Cells were seeded in Ham's F12 medium supplemented with 10% FCS, 100 IU/ml penicillin, and 100 μg/ml streptomycin to a final density of 5×10^5/ml in 55-mm plastic petri dishes (Nunc), and cultured in a 95% air/5% CO_2 incubator at 37°C. Primary, nonconfluent, 5–10 d cultures of chondrocytes were used in all experiments in an attempt to limit changes in gene expression (dedifferentiation). Morphologically, the cells studied were typically flattened with a polygonal cell shape and did not show the fibroblastic appearance of dedifferentiated chondrocytes (Wright et al., 1997). Immunological and molecular analyses confirmed production of similar ECM molecules (type II and VI collagen, fibronectin, and keratin sulphate) and

expression of identical integrin profile subunits (β1, β5αV, α1, α3, and α5) to that of HAC in vivo (Salter et al., 1992; Loeser et al., 1995) and after initial cell extraction (Jopanbutra et al., 1996).

In our experience these chondrocytes show a consistent and reproducible membrane hyperpolarization response to 0.33 Hz mechanical stimulation (Wright et al., 1992, 1996, 1997). We have assessed the electrophysiological response of chondrocytes from knee joint articular cartilage of >80 different individuals and observed no significant difference in the membrane response in cells with respect to gender, age, and cause of death as it relates to patients without a history of locomotor system involvement (unpublished observations).

To investigate whether the membrane hyperpolarization response was critically dependent on IL-4, chondrocytes from the joints of mice heterozygous for IL-4 or IL-4–deficient were studied. Chondrocytes were isolated by sequential enzymatic digestion and cultured as described above. The IL-4 knockout mice used have been previously described (Kopf et al., 1993). Mice were obtained from a colony maintained by Dr. M. Norval (Department of Medical Microbiology, Edinburgh University, Edinburgh, United Kingdom) with permission for use of these animals provided by Professor Horst Bluethmann (Hoffmann-LaRoche AG, Basel, Switzerland).

RNA Extraction

Total RNA was extracted from cultured chondrocytes as described in the micro RNA isolation kit (Stratagene), using a denaturing buffer of 4 M guanidine thiocyanate, 0.75 M sodium citrate, 10% (wt/vol) lauroyl sarcosine, and 7.2 μl/ml β-mercaptoethanol. The quantity of RNA isolated was determined by spectrophotometry using the absorbance reading at 260 nm.

Reverse Transcriptase-PCR (RT-PCR)

Before cDNA synthesis, all RNA samples were incubated with DNase I (Life Technologies) for 15 min in the presence of RNase inhibitor (Life Technologies). Template cDNA was synthesized using 1–5 μg RNA, superscript II, and oligo dT(12–18; Life Technologies) according to the manufacturer's instructions. Primers specific for IL-4 (Arai et al., 1989), IL-4 receptor α (IL-4Rα; Idzerda et al., 1990), the common gamma chain (γc; Takeshita et al., 1992; Puck et al., 1993), and IL-13 receptor α (IL-13Rα; Aman et al., 1996) were used for the PCR reactions: IL-4 5′-TTTGAACAGCCTCACAGAGC-3′, 5′-TCCTTCACAGGACAGGAATT-3′; IL-4Rα 5′-CTTGTTCACCTTTGGACTGG-3′, 5′-CTTGAGCTCTGAGCATTGCC-3′; γc 5′-CTCCTTGCCTAGTGTGGATGG-3′, 5′-CACTGTAGTCTGGCTGCAGAC-3′; and IL-13Rα 5′-GTGAAACATGGAAGACCATC-3′, 5′-GTGAAATAACTGGATCTGATAGGC-3′.

A typical 20-μl PCR reaction contained 16 mM ammonium sulphate, 67 mM Tris/HCl, pH 8.8, 0.01% (vol/vol) Tween 20, 1 μM of each primer, 2 μl cDNA, 100 μM dNTPs, 0.1% (wt/vol) BSA, and 0.25 U *Taq* polymerase (Bioline). The magnesium chloride concentrations for each primer pair were: IL-4, 4 mM; IL-4Rα, 2.5 mM; γc, 2 mM; and IL-13Rα, 1.5 mM. The following program was used for all reactions: 94°C for 3 min; 35 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min 30 s; 72°C for 10 min. PCR products were analyzed by electrophoresis using a 1% (wt/vol) agarose gel.

Cloning and Sequencing

PCR products were cloned into the TA cloning vector (Invitrogen Corp.) as described in the manufacturer's protocol. Each insert was sequenced using the Sanger dideoxy chain termination method (Sanger et al., 1977), modified according to the protocol provided with the sequenase kit (United States Biochemical Corp.).

Mechanical Stimulation of Chondrocytes and Electrophysiological Recording

The technique and apparatus used have been previously described in detail (Wright et al., 1996). For the induction of pressure-induced strain (PIS), 55-mm diameter plastic petri dishes (Nunc) were placed in a sealed pressure chamber with inlet and outlet ports. The chamber was pressurized using nitrogen gas from a cylinder, at a frequency determined by an electronic timer controlling the inlet and outlet valves. The standard stimulation regimen used was a frequency of 0.33 Hz (2 s on/1 s off) for 20 min, 37°C, at a pressure of 16 kPa above atmospheric pressure. This system was shown to produce microstrain on the base of the culture dish (Wright et al., 1996). Membrane potentials of cells were recorded using a single electrode bridge circuit and calibrator, as previously described (Wright et al., 1992; Salter et al., 1997). Microelectrodes with tip resistances of 40–60 MΩ and tip potentials of ~3 mV were used to impale the cells. Membrane potentials of isolated cells were measured and results were accepted if, on cell impalement, there was a rapid change in voltage to the membrane potential level that remained constant for at least 60 s. Experiments were performed at 37°C. The membrane potentials of 5–10 cells were measured before and after the period of PIS.

Anticytokine, antiintegrin, and anticytokine receptor antibodies were added to chondrocytes 30 min before mechanical stimulation. Membrane potentials were measured before and after addition of antibody and after the period of mechanical stimulation. Antibodies had no effect on the resting membrane potential. Antibodies remained in contact with cells during cyclical PIS and when poststimulated membrane potentials were measured. Antibodies against IL-1β, IL-4, IL-4Rα, and γc were obtained from R&D Systems, Inc. Anti–β1 integrin (P4C10) and anti–αVβ5 integrin (P1F6) were obtained from Life Technologies. For each condition tested, at least three experiments were performed on different cells from different donor knees on different days.

Effects of Cytokines on Chondrocyte Membrane Potential

Membrane potential of chondrocytes was measured before and 10 min after the addition of recombinant IL-1β, IL-4, TGF-β1, and interferon gamma (IFN-γ; R&D Systems). To investigate signaling molecules involved in IL-4–induced hyperpolarization chondrocytes were treated, in separate experiments, with a number of pharmacological inhibitors of cell signaling for 30 min before addition of recombinant IL-4. The reagents used (Sigma Chemical Co.) were: neomycin, an inhibitor of PLC (Cockcroft et al., 1985; Kim et al., 1989); flunarizine, an inhibitor of IP3-mediated release of Ca^{2+} from the ER (Seiler et al., 1987); genistein, a tyrosine kinase inhibitor (Akiyama et al., 1987); apamin, a specific blocker of SK channels (Blatz and Magleby, 1986); and gadolinium, a blocker of stretch-activated ion channels (Yang and Sachs, 1989).

Statistics

The mean, SD, and standard error of the mean were determined in each experiment. For statistical comparisons, when the F ratio of the two variances reached significance, the nonparametric Mann-Whitney test was used. When the ratio did not reach significance, the Student's *t* test was used.

Results

A Transferable Factor Induces Membrane Hyperpolarization of HAC in Response to Mechanical Strain

HAC subjected to PIS at 0.33 Hz, 37°C for 20 min undergo hyperpolarization of the plasma membrane by ~45% (Table I). Conditioned medium from mechanically stimulated cells, when added to unstimulated chondrocytes, caused membrane hyperpolarization of these cells similar to that of the directly mechanical strained chondrocytes (Table I), demonstrating the presence of a soluble, transferable factor secreted by the mechanically stimulated chondrocytes. 1 μg/ml P4C10, an anti–β1 integrin antibody, when incubated with chondrocytes for 30 min at 37°C before stimulation, inhibited the hyperpolarization response to mechanical stimulation. Medium from cells mechanically stimulated in the presence 1 μg/ml P4C10, when transferred to unstimulated cells, did not significantly alter the membrane potential of these cells (Table I). In contrast, 1 μg/ml P1F6, an anti–αVβ5 integrin, had no effect on 0.33-Hz cyclical microstrain-induced hyperpolarization or pro-

Table I. Effect on the Membrane Response of Chondrocytes of Direct Stimulation at 0.33 Hz or the Addition of Conditioned Medium (CM) from Chondrocytes Stimulated at 0.33 Hz for 20 min to Previously Unstimulated Cells in a Representative Experiment

Stimulus	Reagent	*n*	% change*		*P*
			Direct	CM	
0.33 Hz PIS	Nil	5	+45	+52	<0.0001
0.33 Hz PIS	P4C10 (anti-β1)	5	−5.3	+0.4	NS
0.33 Hz PIS	P1F6 (anti-αVβ5)	5	+44	+50	0.0009

Results are consistent between experiments and between cells from different individuals for 0.33-Hz stimulation alone (six donors) or with antiintegrin antibodies (three donors).
*Compared with unstimulated cells.

duction of a transferable factor that could induce membrane hyperpolarization of unstimulated chondrocytes.

Cytokines Induce Changes in Membrane Potential

When monolayer cultures of HAC were incubated in separate experiments with a panel of recombinant human cytokines (IL-1β, IFN-γ, TGF-β1, and IL-4), known to be involved in the regulation of chondrocyte metabolism and potentially could function as autocrine/paracrine signaling molecules, a change in membrane potential was seen (Fig. 1). Addition of IL-4 resulted in membrane hyperpolarization, whereas the other cytokines induced membrane depolarization. The effect of IL-4 on the membrane potential of human chondrocytes was dose-dependent over a range between 100 fg/ml and 10 ng/ml. A 17% hyperpolarization response was elicited at concentrations as low as 10 fg/ml and a maximal response was obtained with 5–10 pg/ml (results not shown).

Human Chondrocytes Express IL-4 and IL-4 Receptors

Using immunohistochemical techniques we have shown IL-4 to be present in HAC (Salter et al., 1996). However, its production by these cells and the expression of IL-4 receptors were not previously described. RT-PCR on total RNA isolated from primary cultured chondrocytes using primers specific for IL-4 resulted in amplification of a 269-bp region of DNA (Fig. 2). This DNA region, when cloned and sequenced, displayed 100% identity to the published sequence of human lymphocyte IL-4 mRNA (Arai et al., 1989). RT-PCR reactions using primers to IL-4Rα, γc, and IL-13Rα revealed DNA products of 465, 356, and 450 bp, respectively (Fig. 2), corresponding to the components of both the type I IL-4 receptor (IL-4Rα/γc) and type II receptor (IL-4Rα/IL-13Rα).

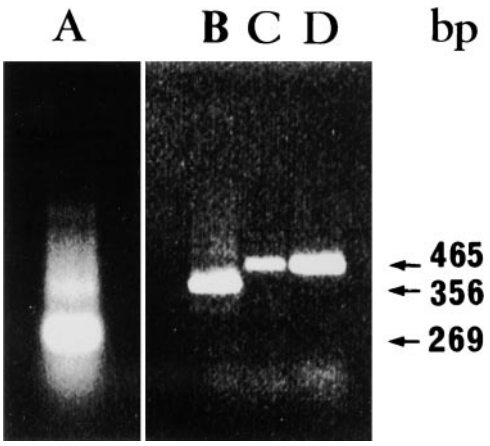

Figure 2. Agarose gel electrophoresis of PCR products amplified from reverse-transcribed RNA of normal chondrocytes. The following are in lanes A, B, C, and D, respectively: IL-4, γc, IL-4Rα, and IL-13Rα. Sizes of products shown are in basepair. Results are consistent between experiments and between cells from four different donors.

IL-4 Is Necessary for the Membrane Hyperpolarization Response to Mechanical Stimulation

Neutralizing antibodies to IL-4 abolished the hyperpolarization response to cyclical strain, whereas neutralizing antibodies to IL-1β had no effect (Fig. 3). Specific antibodies to IL-4Rα (10 μg/ml) prevented the hyperpolarization response of chondrocytes to mechanical stimulation, whereas inhibitory antibodies to the γc subunit had no effect on the response (Fig. 3). Anti–IL-4 antibodies (1 μg/ml), added to medium after mechanical stimulation but before transfer of that medium to unstimulated cells, prevented subsequent hyperpolarization of unstained cells (Table II).

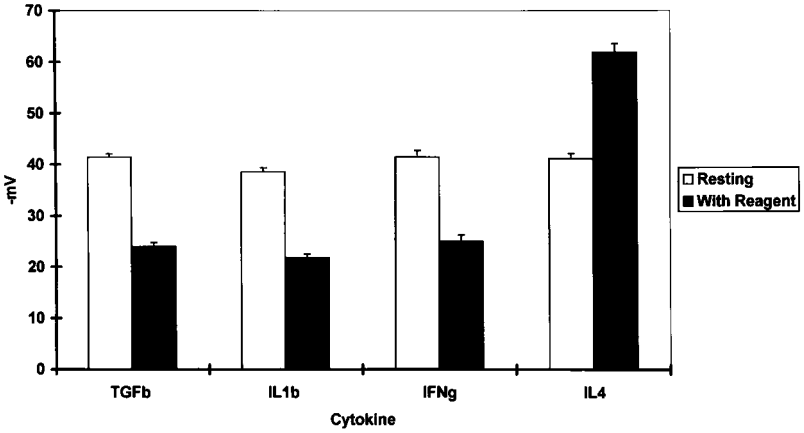

Figure 1. Effect of the addition of TGF-β1, IL-1β, IFN-γ, or IL-4 (10 ng/ml) on the membrane potential of mechanically unstimulated chondrocytes ($n = 10$; TGF-β1, $P < 0.0001$; IL-1β, $P < 0.0001$; IFN-γ, $P < 0.0001$; IL-4, $P < 0.0001$). Results are consistent between experiments and between cells from different individuals for IL-4 alone (18 donors), TGF-β1, IL-1β, and IFN-γ (3 donors).

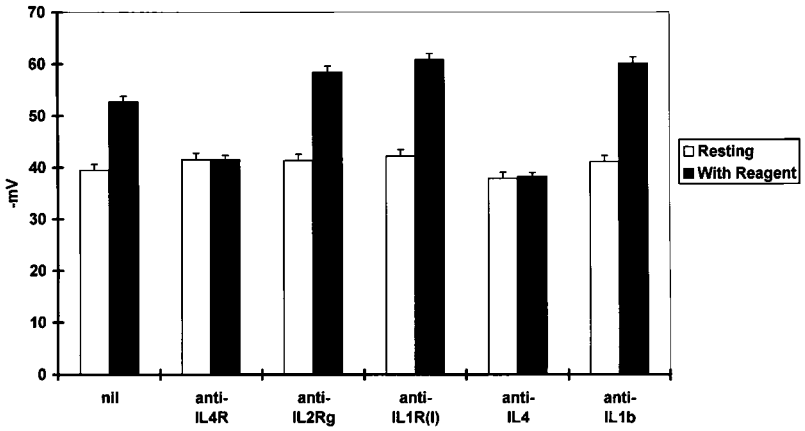

Figure 3. Effect of addition of antibodies to cytokines or cytokine receptors on the membrane hyperpolarization response of HAC to 0.33-Hz mechanical stimulation ($n = 5$; control, $P < 0.0001$; anti-γc, $P < 0.0001$; anti–IL-1RI, $P < 0.0001$; anti–IL-1β, $P < 0.0001$). Results shown are from a single experiment and are consistent between experiments and between cells from different individuals for anti–IL-4 (7 donors), anti–IL-1β (3 donors), anti-γc (3 donors), anti–IL-4Rα (3 donors), and anti–IL-1RI (3 donors).

Chondrocytes isolated from the articular cartilage of knee joints from IL-4 knockout mice did not show a significant change in membrane potential after 20 min of mechanical stimulation at 0.33 Hz (Fig. 4). In contrast, chondrocytes isolated from knee joints of heterozygous mice showed a similar hyperpolarization response (Fig. 4) to mechanical stimulation as that seen with HAC.

IL-4–mediated Membrane Hyperpolarization Involves PLC and IP3

The hyperpolarization response of HAC to recombinant human IL-4 (10 pg/ml) was unaffected by P4C10 (anti–β1 integrin), genistein (a tyrosine kinase inhibitor), and gadolinium (a blocker of mechanosensitive ion channels; Table III), although these agents were shown previously to inhibit the hyperpolarization response of HAC to mechanical stimulation (Wright et al., 1996, 1997). Neomycin (an inhibitor of PLC), flunarizine (an inhibitor of IP3-mediated release of Ca^{2+} from the ER), and apamin (an SK channel blocker) each inhibited the chondrocyte hyperpolarization response to IL-4 (Table III).

Discussion

This study has shown for the first time that IL-4 and its receptor are expressed by HAC. Furthermore this study also has shown that the cytokine receptor pair are involved in the integrin-dependent signaling pathway activated by 0.33-Hz cyclical strain that leads to the opening of SK channels and membrane hyperpolarization.

Close associations between integrin and growth factor–mediated signaling in regulation of cell function are being identified. Cell adhesion–dependent activation of the Ras/MAPK pathway may involve tyrosine phosphorylation of PDGF receptors (Sundberg and Rubin, 1996). Angiogenic effects of a number of growth factors including basic fibroblast growth factor and vascular endothelial growth factor are integrin-regulated (Friedlander et al., 1995). Integrin-mediated cell adherence also has been shown to be important in cytokine gene expression in synovial fluid cells from patients with rheumatoid arthritis (Miyake et al., 1993) and by mast cells after Ig E receptor aggregation (Ra et al., 1994). Wilson et al. (1993) have demonstrated previously that mechanical strain induces growth of vascular smooth muscle cells via an autocrine action of PDGF. However, the growth-promoting effect required 36–48 h of mechanical stimulation and was associated with increased levels of PDGF mRNA, suggesting slow production and release of the cytokine rather than the rapid release of a preformed mediator after mechanical stimulation, as demonstrated in our system.

It is unclear how integrin-mediated signaling causes IL-4 release. Rapid release of neurotransmitter from frog mus-

Table II. Effect of IL-4 Antibody in CM on the Membrane Hyperpolarization Response of Chondrocytes

Stimulus	Reagent	Dish	*n*	% change*	*P*
0.33 Hz PIS	Nil	A	5	+45	<0.0001
CM	Nil	B	5	+52	<0.009
0.33 Hz PIS	Nil	C	5	+54	<0.0001
CM	Anti–IL-4	D	5	−0.01	NS

Chondrocytes in dishes A and C were stimulated at 0.33 Hz PIS and the CM transferred to dishes B and D, respectively. Before the addition of CM from dish C to D, IL-4 antibody was added at a concentration of 1 μg/ml. Results are from a single experiment and are consistent between experiments and between cells from different individuals for transfer in the absence (six donors) or presence of anti–IL-4 antibody (three donors).
*Compared with unstimulated cells.

Table III. Effect of Chemical Inhibitors of Cell Signaling Molecules on the Membrane Hyperpolarization Response of HAC Treated with 10 pg/ml IL-4

Stimulus	Reagent	*n*	% change*	*P*
IL-4	Nil	5	+30.2	<0.0001
IL-4	Apamin (4.9 μM)	5	+0.01	NS
IL-4	Flunarizine (50 μM)	5	−0.01	NS
IL-4	Gadolinium (10 μM)	5	+31.3	<0.0001
IL-4	Genistein (40 μM)	5	+50.5	<0.0001
IL-4	Neomycin (5 mM)	5	−0.03	NS
IL-4	P4C10 (1 μg/ml)	5	+66	<0.0001

Neomycin (inhibitor of PLC), flunarizine (inhibitor of IP3-mediated Ca^{2+} release from ER), apamin (blocker of SK channels), genistein (tyrosine kinase inhibitor), gadolinium (blocker of mechanosensitive ion channels), and P4C10 (anti–β1 integrin). Results shown are from a single experiment and are consistent between experiments and between cells from different individuals for IL-4 alone (18 donors), IL-4 + apamin (3 donors), IL-4 + either flunarizine (3 donors), gadolinium (3 donors), neomycin (3 donors), or P4C10 (3 donors).
*Compared with unstimulated cells.

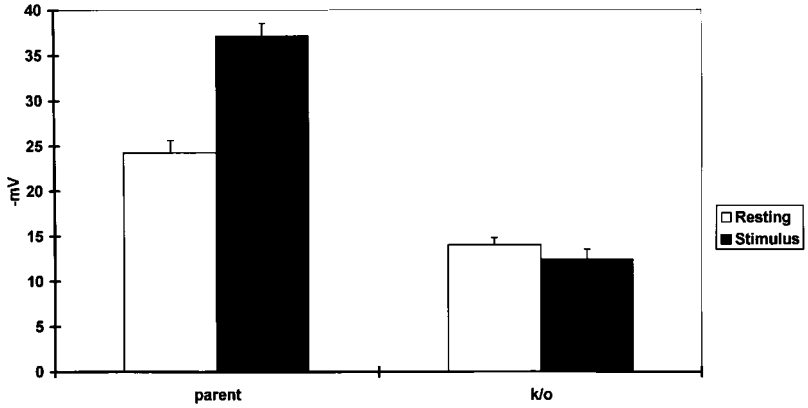

Figure 4. Effects of PIS on the membrane potentials of chondrocytes isolated from the articular cartilage of parent and IL-4 knockout mice (n = 5; parent: $P < 0.0002$). Representative results are from three separate experiments.

cle motor nerve terminals after stretch is integrin-dependent and requires both intra and extracellular calcium (Chen and Grinnell, 1995). The data from our studies suggest that mechanical stimulation induced release of IL-4 by human chondrocytes after recognition and transduction of the mechanical signal by $\alpha5\beta1$ integrin. Furthermore, activation of a signaling pathway involving tyrosine kinases, stretch-activated ion channels, and the actin cytoskeleton is consistent with other models of integrin-mediated mechanotransduction (Glogauer et al., 1997; Maniotis et al., 1997; Muller et al., 1997, Schmidt et al., 1998). IL-4 in turn binds to the chondrocyte IL-4 receptor heterodimer, IL-4Rα/IL-13Rα, initiating a signal cascade involving PLC and IP3-mediated Ca^{2+} release and subsequent activation of SK channels, leading to K^+ efflux and membrane hyperpolarization.

Coordinated activations of integrin and IL-4–associated signaling pathways in chondrocytes are of potential importance in regulating the structure and function of normal and diseased articular cartilage. Regulation occurs by mediating other biochemical responses to mechanical strain, e.g., proteoglycan synthesis (Veldhuijzen et al., 1979), or altering the expression of other ECM proteins, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs) involved in the pathogenesis of OA (Dean, 1991). Studies of cytokine effects on chondrocytes in vitro suggest that IL-4 alters the ratio of MMPs and TIMPs in favor of TIMPs by suppressing IL-1–stimulated MMP3 production (Shingu et al., 1995; Nemoto et al., 1997). Integrin-regulated production of IL-4, as a result of optimal mechanical stimulation in normal articular cartilage in vivo, would be chondroprotective by inhibiting cartilage degradation and promoting matrix synthesis in normal articular cartilage. In contrast, in joint diseases such as OA, normal mechanotransduction pathways may be disrupted following changes in integrin expression by chondrocytes (Lapadula et al., 1997) or neo-expression of adhesive and antiadhesive molecules such as fibronectin (Chevalier et al., 1996) and tenascin (Salter, 1993) in the pericellular matrix, resulting in abnormal chondrocyte activity. Indeed, preliminary data from our laboratory indicate that chondrocytes from OA cartilage show an abnormal electrophysiological response to both mechanical stimulation and direct application of IL-4 (Wright et al., 1998). Further elucidation of the signaling events activated by mechanical stimuli in HAC from normal and diseased cartilage should lead to a better understanding of how cartilage is maintained by mechanical stimuli in health and disease. These studies suggest that better understanding of the signaling molecules involved in mechanotransduction in chondrocytes may also lead to the identification of novel targets for therapy in OA.

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