Biorheology 39 (2002) 153–160
IOS Press

The functional expression of connexin 43 in articular chondrocytes is increased by interleukin 1β: Evidence for a Ca^{2+}-dependent mechanism

Rossana Tonon and Paola D'Andrea
Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università di Trieste, via Licio Giorgieri 1, I-34127 Trieste, Italy
Tel.: +39 40 676 3690; Fax: +39 40 676 3691; E-mail: dandrea@bbcm.univ.trieste.it

Abstract. Cell-to-cell interactions and gap junctions-dependent communication are crucially involved in chondrogenic differentiation, while in adult articular cartilage direct intercellular communication occurs mainly among chondrocytes facing the outer cartilage layer. Chondrocytes extracted from adult articular cartilage and grown in primary culture express connexin 43 and form functional gap junctions capable of sustaining the propagation of intercellular Ca^{2+} waves. Degradation of articular cartilage is a characteristic feature of arthritic diseases and is associated to increased levels of interleukin-1 (IL-1) in the synovial fluid. We have examined the effects of IL-1 on gap junctional communication in cultured rabbit articular chondrocytes. Incubation with IL-1 potentiated the transmission of intercellular Ca^{2+} waves and the intercellular transfer of Lucifer yellow. The stimulatory effect was accompanied by a dose-dependent increase in the expression of connexin 43 and by an enhanced connexin 43 immunostaining at sites of cell-to-cell contact. IL-1 stimulation induced a dose-dependent increase of cytosolic Ca^{2+} and activates protein tyrosine phosphorylation. IL-1-dependent up-regulation of connexin 43 could be prevented by intracellular Ca^{2+} chelation, but not by inhibitors of protein tyrosine kinases, suggesting a crucial role of cytosolic Ca^{2+} in regulating the expression of connexin 43. IL-1 is one of the most potent cytokines that promotes cartilage catabolism: its modulation of intercellular communication represents a novel mechanism by which proinflammatory mediators regulate the activity of cartilage cells.

1. Introduction

Intercellular communication confers tissues the ability to co-ordinate many different cellular functions, such as the regulation of cell volume, the intracellular ionic composition and cell metabolism [3]. Cell-to-cell communication through gap junctions represents the pathway for direct intercellular exchange of small cytosolic constituents that diffuse through the intercellular channels constituted by the multimeric assembly of connexins, a family of closely related proteins [3].

Gap junction-mediated intercellular communication is critically involved in the development of cartilage during differentiation [6]; in adult articular cartilage, on the other hand, chondrocytes exist as individual cells embedded in the extracellular matrix, and gap junctions are mainly expressed by the flattened chondrocytes facing the outer cartilage layer [24]. Chondrocytes extracted from adult articular cartilage, however, retain the ability to form functional gap junctions in culture, as demonstrated by the expression of connexin 43 (Cx43), the 43 kD gap junction protein [4,8], and by the ability to respond to extracellular chemical or mechanical stimuli with co-ordinated patterns of intercellular Ca^{2+} oscillations and waves [7,12].

0006-355X/02/$8.00

Degradation of articular cartilage is a characteristic feature of arthritic diseases and results from increased levels of matrix metalloproteinases such as collagenase and stromelysin [18]. Interleukin-1 (IL-1) is one of the most potent cytokines that promotes cartilage catabolism; its levels are elevated in arthritic joints and induce in chondrocytes the production of metalloproteinases [26], suppresses type II collagen and proteoglycan production [11], inhibits chondrocyte proliferation [2] and induces proinflammatory mediators such as prostaglandins and nitric oxide [18]. Interestingly, chondrocytes from the outer cartilage layer show a greater vulnerability to the harmful effects of IL-1 than cells in the deeper layers of the tissue [13].

Among the articular pathologies linked to increased IL-1 levels, rheumatoid arthritis induce an unscheduled remodelling of cartilage and the degradation of extracellular matrix [1]. As a part of synovial tissue reaction, proliferating synovial cells penetrate the superficial cartilage layer in the form of a pannus, causing the destruction of cartilage [5]. Synovial pannus formation is initiated with the recognition and adhesion of synovial cells to chondrocytes and to cartilage matrix [15,22,23,27]. Studies *in vitro* demonstrated that IL-1 stimulates dose-dependently the attachment of monocytes and synovial cells to cartilage through a mechanism involving an increased and altered expression of integrins and cadherins [16].

There is growing evidence that intercellular adhesion precedes, and may even be a prerequisite for, the establishment of gap junctions [17,20]. Moreover, evidence has been reported that inflammatory diseases in kidneys and lungs are associated with an increased expression of Cx43, resulting in a higher level of cell-to-cell coupling [9,14].

In this study we show that stimulation of rabbit articular chondrocytes in primary culture with IL-1β induces a dose-dependent up-regulation of Cx43. Increased Cx43 levels are associated with a higher level of intercellular dye coupling and with a potentiation of mechanically-induced intercellular Ca^{2+} waves, indicating that IL-1 enhances intercellular communication and signalling in articular cartilage. IL-1 induces dose-dependent increases of the cytosolic Ca^{2+} concentration and protein tyrosine phosphorylation. Intracellular Ca^{2+} chelation totally prevents IL-1-dependent Cx43 up-regulation, while tyrosine kinases inhibitors did not affect the level of IL-1β-induced Cx43 expression: the action of the cytokine on intracellular communication appears therefore mediated cytosolic Ca^{2+} increases.

2. Results

Chondrocytes extracted from rabbit articular cartilage and grown in primary cultures for 6–7 days form confluent layers of 40–60 cells; complete confluence is normally attained 1–2 days later. The intracellular Ca^{2+} concentration of chondrocytes under resting conditions was 92 ± 11 nM ($n = 368$), as measured by digital video imaging in cells loaded with fura-2.

We showed in previous studies [4,12] that mechanical stimulation of a single cell, obtained by briefly distorting the plasmamembrane with a fire-polished glass micropipette, induced a rapid increase of the cytosolic Ca^{2+} concentration to 1957 ± 270 nM ($n = 368$) followed by a subsequent decline to resting levels. Besides inducing a Ca^{2+} rise in the stimulated cell, mechanical stimulation gave rise to intercellular Ca^{2+} waves which propagated radially from the point of stimulation and involved 10 ± 2 chondrocytes ($n = 57$). We recently demonstrated that Ca^{2+} wave propagation depends on the intercellular diffusion of $InsP_3$ through gap junctions, followed by the intracellular Ca^{2+} release in neighbouring cells [12]. Given the pleiotropic effects induced by IL-1 on cartilage cells and its role in promoting cellular interactions at the chondro-synovial junction, we tested possibility that the cytokine could modulate also

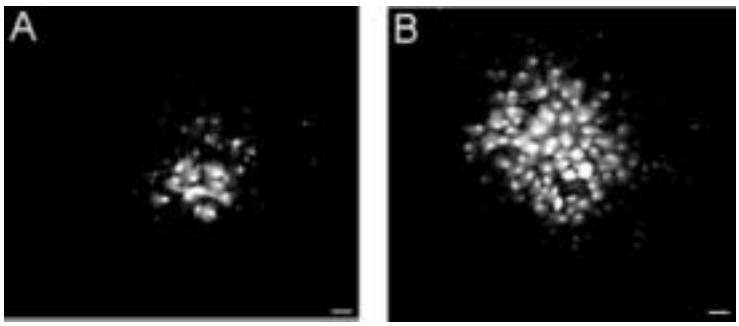

Fig. 1. IL-1β increases the extent of dye coupling. Intercellular dye transfer was measured in confluent chondrocytes cultures by microinjection of Lucifer yellow. Fluorescence micrographs were taken 8 min after microinjection of Lucifer yellow into one cell. Injections were performed before (A) or after incubation with 10^{-8} M IL-1β for 24 h (B). Bar: 40 μm.

the level of cell-to-cell coupling among articular chondrocytes. Incubation of cells for 24 h with 10^{-8} M IL-1β resulted in an increased propagation of the mechanically-induced intercellular Ca^{2+} wave which diffused to higher distance and involved 24 ± 3 cells ($n = 22$).

The extent of intercellular coupling was assessed in confluent cell layers by microinjecting individual cells with Lucifer yellow and measuring the intercellular dye transfer (Fig. 1). In control cultures the intercellular diffusion of Lucifer yellow was observed in 14 ± 1.5 cells ($n = 7$) (Fig. 1A), while in cultures treated with 10^{-8} M IL-1β (24 h) the number of fluorescence-labelled cells was 37 ± 3 ($n = 18$) (Fig. 1B), indicating that the cytokine increased the degree of cell-to-cell coupling.

Among the most widely connexins expressed, articular chondrocytes express elevated levels of Cx43, but not Cx32 or Cx26 [4,8]. Indirect immunofluorescence using a commercially available anti Cx43 antibody revealed a punctate distribution of Cx43 immunoreactivity particularly abundant at the interface of adjoining cells (Fig. 2). With respect to control cultures (Fig. 2A), cell treated for 24 h with increased concentrations of IL-1β (5×10^{-11} M, Fig. 2B; 5×10^{-10} M, Fig. 2C and 10^{-8} M, Fig. 2D) show a dose-dependent increase of Cx43 immunoreactivity. Higher cytokine doses (up to 10^{-7} M) failed to further increase the level of staining (not shown).

Immunoblotting experiments demonstrated the expression of four bands on chondrocyte cell lysates (Fig. 3) corresponding to the unphosphorylated (NP) and to differently phosphorylated isoforms of Cx43 (P1, P2, P3) [21]. Cell stimulation with increasing concentrations of IL-1β (5×10^{-11} M, lane 2; 5×10^{-10} M, lane 3, 5×10^{-9} M, lane 4 and 10^{-8} M, lane 5) for 6 h (Fig. 3, upper panel) or 24 h (Fig. 3, lower panel) induced a dose-dependent up-regulation of all Cx43 bands with respect to controls (lane 1). At both incubation times, the maximal stimulation of Cx43 expression was obtained with IL-1β 10^{-8} M, while higher cytokine doses (up to 10^{-7} M) did not stimulate further Cx43 increase (not shown). The concentration of IL-1β 10^{-8} M was therefore employed in all subsequent experiments.

In time–course experiments, an increase of Cx43 expression was detected after 6 h stimulation ($166 \pm 15\%$, $n = 3$), although a slight increase could sometimes be observed after 30 min (see below). Stimulation for 24 h maximally increased the level of Cx43 ($180 \pm 13\%$, $n = 3$) while prolonged stimulation times (up to 48 h) failed to induce a further up-regulation (not shown).

In a next series of experiments we investigated the possible mechanism responsible for the increased expression of Cx43 induced by IL-1β. In articular chondrocytes IL-1β has been shown to induce dose-dependent increases of the cytosolic Ca^{2+} concentration [19]. Although the transduction mechanism has been not fully elucidated, a necessary role of focal adhesions has been suggested.

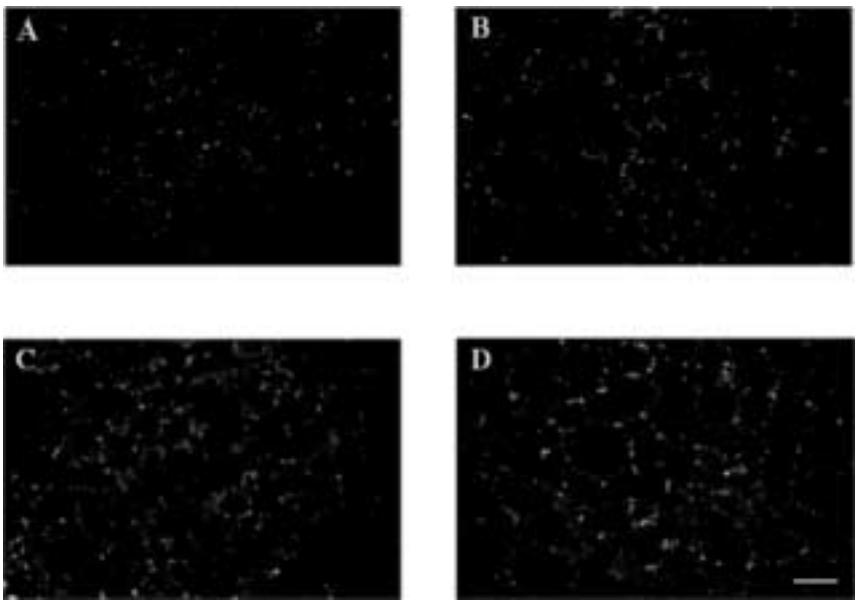

Fig. 2. IL-1β increases Cx43 immunoreactivity. Confluent chondrocytes cultures were stimulated, fixed, permeabilized and incubated with a polyclonal antibody to Cx43. A FITC-coniugated secondary antibody was used for fluorescence visualisation. Under control conditions (A) chondrocytes exhibit a punctate staining pattern that represents gap junctions composed of Cx43 located primarily in the plasma membrane between adjacent cells. Stimulation of the cells for 24 h with IL-1β 5×10^{-11} M (B), 5×10^{-10} M (C) and 10^{-8} M (D) increased dose-dependently the abundance of the punctuate staining. Bar: 40 μm.

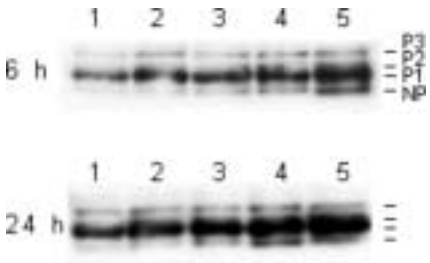

Fig. 3. IL-1β induces dose-dependent increases of Cx43 protein levels. Cells grown to confluence were incubated with increasing concentrations of IL-1β for 6 h (A) or 24 h (B). The protein extracts (10–15 μg for each sample) were separated by SDS polyacrylamide gel electrophoresis, transferred to nitro-cellulose and analysed using a polyclonal antibody to rat Cx43. Control (lane 1), IL-1β 5×10^{-11} M (lane 2), 5×10^{-10} M (lane 3), 5×10^{-9} (lane 4) and 10^{-8} M (lane 5). Multiple bands corresponding to phosphorylated (P1, P2, and P3) and unphosphorylated (NP) forms of Cx43 are present.

Stimulation of confluent cell monolayers with IL-1β induced dose-dependent increases of the cytosolic Ca^{2+} concentration (Fig. 4). At lower cytokine doses (5×10^{-11} M, Fig. 5A, 5×10^{-10} M, Fig. 4B) the increase was slow and long lasting (reaching 129 ± 18 nM, $n = 12$ and 133 ± 22 nM, $n = 9$, respectively), while at the maximal doses employed (10^{-8} M, Fig. 4C) the cell response appeared biphasic and composed by an initial spike (reaching 250 ± 30 nM, $n = 15$) followed by a sustained plateau (120 ± 5 nM, $n = 15$). The cytokine-induce Ca^{2+} response could be prevented by preincubating the cells (1 h) with the membrane-permeant Ca^{2+} chelator BAPTA-AM (25 μM, Fig. 4D).

Another important signal transduction pathway stimulated in chondrocytes by IL-1β is the activation of tyrosine kinases [10]. We, therefore, tested the ability of IL-1β to induce protein tyrosine phos-

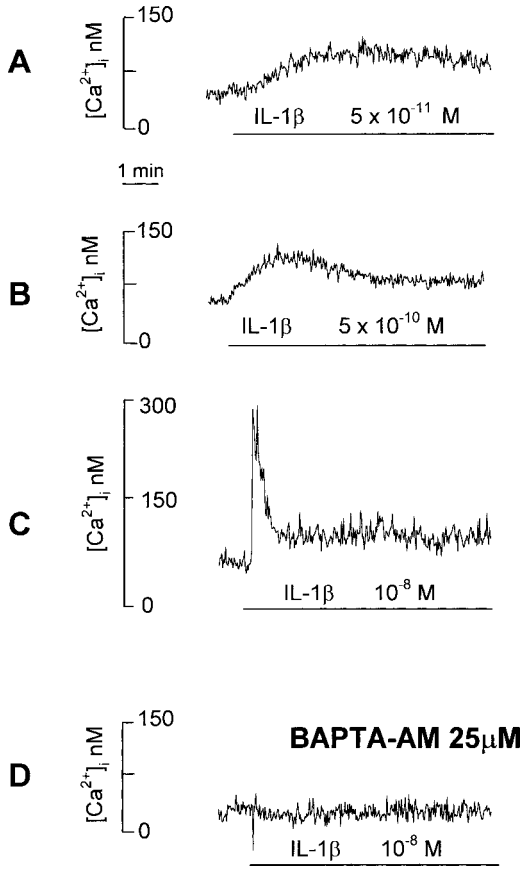

Fig. 4. IL-1β induces dose-dependent increases of the cytosolic Ca^{2+} concentration. Changes in intracellular Ca^{2+} were monitored by calcium imaging in cells loaded with fura-2. For each series of experiments is reported a representative tracing. (A–C) Cells were stimulated with indicated dosages of IL-1β. (D) Cells were preincubated with BAPTA-AM 25 μM for 1 h prior to stimulation with IL-1β 10^{-8} M.

phorylation in our chondrocyte cultures. Time-dependent activation of protein tyrosine phosphorylation was analysed in Western blots of whole cell lysates probed with an anti-phosphotyrosine antibody (not shown). IL-1β 10^{-8} M induced the tyrosine phosphorylation of several proteins migrating at 64, 53, 35 and 33 kDa. Tyrosine phosphorylation of these proteins was evident after 5 min stimulation and was maintained in cells stimulated for 24 h.

In order to verify whether the effects of IL-1β on Cx43 expression and protein tyrosine phosphorylation could derive from the increased Ca^{2+} levels induced by the cytokine, we assayed the expression of Cx43 in cells stimulated in the presence or in the absence of BAPTA-AM 25 μM, the concentration effective in preventing the rise of cytosolic Ca^{2+} induced by maximal IL-1β doses (10^{-8} M). Cell pre-treatment with the chelator alone (1 h) did not appreciably altered the level of Cx43 compared to controls (Fig. 5A) nor the basal tyrosine phosphorylation of cellular proteins (Fig. 5B). The chelator, however, consistently inhibited the up-regulation of Cx43 induced by IL-1β. The effect could be already detected in cells stimulated with the cytokine for 30 min, and fully evident in cells stimulated for 6 h (Fig. 5A). Cell pre-treatment with BAPTA-AM appeared effective also in preventing protein tyrosine phosphorylation induced by IL-1β (Fig. 5B).

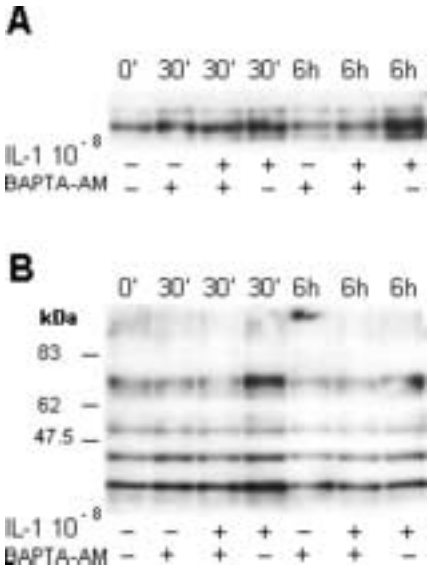

Fig. 5. BAPTA-AM prevents the effects of IL-1β on Cx43 expression and protein tyrosine phosphorylation. (A) Cells grown to confluence were incubated with IL-1β 10^{-8} M for the times indicated. In parallel experiments, cells were incubated for 1 h with 25 μM BAPTA-AM prior to cytokine stimulation. The protein extracts (10–15 μg for each sample) were separated by SDS polyacrylamide gel electrophoresis, transferred to nitro-cellulose and analysed using the polyclonal anti-Cx43. (B) 20 μg of the same protein extracts were analysed using a monoclonal antibody to phosphotyrosine (4G10).

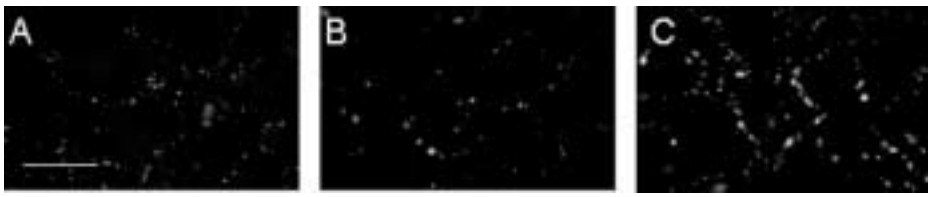

Fig. 6. BAPTA-AM prevents the effects of IL-1β on Cx43 immunolocalization. Confluent chondrocytes cultures were fixed, permeabilized and incubated with a polyclonal antibody to Cx43 in resting conditions (A), after incubation either with BAPTA-AM 25 μM for 1 h prior to stimulation with IL-1β 10^{-8} M for 6 h (B), or after stimulation with IL-1β 10^{-8} M for 6 h (C). A FITC-coniugated secondary antibody was used for fluorescence visualisation. Bar: 40 μm.

In another set of experiments stimulation with IL-1β was carried out in the presence and in the absence of tyrosine kinases inhibitors, to test the effects of protein tyrosine phosphorylation on Cx43 expression. Cell pretreatment with herbimycin A (1 μM, 2 h) did not prevent the IL-1β-induced Cx43 up-regulation, and did not interfere with the inhibitory action of BAPTA-AM, suggesting that tyrosine phosphorylation is not directly involved in the signalling pathway leading to the increased expression of Cx43 (not shown). Similar results were obtained preincubating the cells in the presence of tyrphostin A 25 (100 μM, 15 min, not shown).

Immunofluorescence experiments were carried out to verify whether the treatment with BAPTA-AM induced major modifications in the cellular localisation of Cx43 (Fig. 6). The punctate distribution of Cx43 immunoreactivity, evident in control cultures (Fig. 6A) was not altered in cells pre-treated with BAPTA-AM and stimulated with 10^{-8} M IL-1β for 6 h (Fig. 6B). Moreover, the average level of staining was similar in both conditions. In the absence of BAPTA-AM, cell stimulation with 10^{-8} M IL-1β greatly enhanced the Cx43-specific staining at appositional membranes (Fig. 6C).

3. Conclusions

In this study, we examined the effect of the proinflammatory cytokine IL-1β on gap junction-mediated intercellular communication in articular chondrocytes. We found that the cytokine increases dose-dependently the expression of Cx43, its localisation at the plasmamembrane, the extent of dye transfer and the propagation of mechanically-induced intercellular Ca^{2+} waves, indicating a positive modulation of cell-to-cell coupling. Among the intracellular signals activated by IL-1β in these cells, an increase of cytosolic Ca^{2+} appears necessarily required, since preventing Ca^{2+} rises with BAPTA-AM completely abolished the cytokine-induced Cx43 up-regulation.

Modulation of intercellular coupling represents a novel aspect of cartilage response to proinflammatory signals and is likely to have important consequences in cartilage pathology. Inflammatory and degenerative joint diseases are invariably associated with elevated levels of IL-1, one of the most important mediators responsible for cartilage matrix catabolism [18]. IL-1-induced chondrocytes responses represent central pathogenic events in rheumatoid arthritis and osteoarthritis and include the induction of metalloproteinases, of other proinflammatory cytokines and the inhibition of both extracellular matrix synthesis and chondrocyte proliferation [18]. The resulting dysregulation of cartilage homeostasis leads to progressive destruction of the joints.

The IL-1-dependent up-regulation of Cx43 expression, reported in this study, appears to be associated to the activation of Ca^{2+} signalling, since it is prevented by intracellular Ca^{2+} chelation. Interestingly, this condition prevents also part of the cytokine-induced protein tyrosine phosphorylation, suggesting that IL-1 activates multiple tyrosine kinases in cartilage cells. The activation of Ca^{2+} signalling by IL-1 is strictly dependent on focal adhesions [19]: a likely candidate for a direct linkage between Ca^{2+} rises and tyrosine phosphorylation could be the Ca^{2+}-dependent tyrosine kinase Pyk2, a member of the focal adhesion kinase family which can be activated by stress signals [25]. In this context, chondrocyte response to proinflammatory stimuli would depend on the adhesive status of the cells, which is deeply modified in pathological cartilage: the erosion of the matrix embedding individual cells leads to the direct exposition of chondrocytes to novel cell–matrix and cell-to-cell interactions, and in rheumatoid arthritis, to the direct interaction with the infiltrating inflammatory cells [5].

Gap junctions-mediated intercellular communication provide a pathway for a bi-directional transfer of ions and small molecules between the cells; their permeability to intracellular second messengers confers tissues the ability to respond uniformly to localised stimuli [3]. Enhanced communication competence among chondrocytes and, possibly, between chondrocytes and synovial cells, may facilitate the diffusion of locally generated signals, such as mechanical strain, and thus regulate the sensitivity of cartilage and synovial pannus to hormonal and physical factors.

Acknowledgements

We would like to thank Franco Vittur for support and helpful discussions. The work was supported by MURST-PRIN2000 and by University of Trieste, Italy.

References

[1] W.P. Arend, The pathophysiology and treatment of rheumatoid arthritis, *Arthritis Rheum*. **40** (1997), 595–597.
[2] F. Blanco and M. Lotz, IL-1 induced nitric oxide inhibits chondrocyte proliferation via PGE2, *Exp. Cell Res*. **218** (1995), 319–325.

[3] R. Bruzzone, T.W. White and D.L. Paul, Connections with connexins: the molecular basis of direct intercellular signalling, *Eur. J. Biochem*. **238** (1996), 1–27.
[4] I. Capozzi, R. Tonon and P.D. Andrea, Ca^{2+}-sensitive phosphoinositide hydrolysis is activated in synovial cells but not in articular chondrocytes, *Biochem. J.* **344** (1999), 545–553.
[5] M.W.K. Chew, B. Henderson and J.C.W. Edwards, Antigen-induced arthritis in rabbit: ultrastructural changes at the chondrosynovial junction, *Int. J. Exp. Path*. **71** (1990), 879–894.
[6] C.N.D. Coelho and R.A. Kosher, Gap junctional communication during limb cartilage differentiation, *Dev. Biol*. **144** (1991), 47–53.
[7] P. D'Andrea and F. Vittur, Gap junctions modulate intercellular Ca^{2+} signalling in cultured articular chondrocytes, *Cell Calcium* **20** (1996), 389–397.
[8] H. Donahue, F. Guilak, M.A. Vander Moolen, K.J. McLeod, C.T. Rubin, D.A. Grande and P.R. Brink, Chondrocytes isolated from mature articular cartilage retain the capacity to form functional gap junctions, *J. Bone Min. Res*. **10** (1995), 1359–1364.
[9] M. Fernandez-Cobo, C. Gingalewski and A. Di Maio, Expression of the connexin43 gene is increased in the kidney and the lungs of rats injected with bacterial lipopolysaccharide, *Shock* **10** (1998), 97–102.
[10] Y. Geng, J. Valbracht and M. Lotz, Selective activation of the mitogen-activated protein kinase subgroups c-Jun NH sub 2 terminal kinase and p38 by IL-1 and TNF in human articular chondrocytes, *J. Clin. Invest*. **98** (1996), 2425–2430.
[11] M.B. Goldring, J.R. Brikhead, L.F. Suen, R. Yamin, S. Mizuno, J. Glowacki, J.L. Arbiser and J.F. Apperley, Interleukin-1β-modulated gene expression in immortalised human chondrocytes, *J. Clin. Invest*. **94** (1994), 2307–2316.
[12] M. Grandolfo, A. Calabrese and P. D'Andrea, Mechanism of mechanically-induced intercellular calcium waves in rabbit articular chondrocytes and in HIG-82 synovial cells, *J. Bone Min. Res*. **13** (1998), 443–453.
[13] H.J. Häuselmann, J. Flechtenmacher, L. Michal, E. Thonar, M. Shinmei, K.E. Kuettne and M.B. Aydelotte, The superficial layer of human articular cartilage is more susceptible to interleukin-1-induced damage than deep layers, *Arthritis Rheum*. **39** (1996), 478–488.
[14] G.S. Hillis, L.A. Duthie, P.A.J. Brown, J.G. Simpson, A.M. Macleod and N.E. Haites, Upregulation and co-localization of connexin43 and cellular adhesion molecules in inflammatory renal diseases, *J. Pathol*. **182** (1997), 373–379.
[15] H. Ishikawa, S. Hirata, Y. Nishibayashi, S. Imura, H. Kubo and O. Ohno, The role of adhesion molecules in synovial pannus formation in rheumatoid arthritis, *Clin. Orthop*. **300** (1994), 297–303.
[16] H. Ishikawa, O. Ohno, R. Sura, T. Matsubara and K. Hirohata, Cytokine enhancement of monocyte/synovial cell attachment to the surface of cartilage: a possible trigger of pannus formation in arthritis, *Rheumatol. Int*. **11** (1991), 31–36.
[17] W.M.F. Jongen, D.J. Fitzgerald, M. Asamoto et al., Regulation of connexin43-mediated gap junctional intercellular communication by Ca^{2+} in mouse epidermal cells is controlled by E-cadherin, *J. Cell Biol*. **114** (1991), 545–556.
[18] M. Lotz, F.J. Blanco, J. von Kempis, J. Dudler, R. Maier, P.M. Villiger and Y. Geng, Cytokine regulation of chondrocyte functions, *J. Rheumatol*. **22** (1995), 104–108.
[19] L. Luo, T. Cruz and C. McCulloch, Interleukin 1-induced calcium signalling in chondrocytes requires focal adhesions, *Biochem. J*. **324** (1997), 653–658.
[20] R.-M. Mege, F. Matsuzaki, W.J. Gallin, J.I. Goldberg, B.A. Cunningham and G.M. Edelman, Construction of epithelioid sheets by transfection of mouse sarcoma cells with cDNAs for chicken adhesion molecules, *Proc. Natl. Acad. Sci. USA* **85** (1988), 7274–7278.
[21] A.P. Moreno, J.C. Sàez, G.I. Fishman and D.C. Spray, Human connexin43 gap junction channels: regulation of unitary conductances by phosphorylation, *Circ. Res*. **74** (1994), 1050–1057.
[22] A. Ramachandrula, K. Tiku and M.L. Tiku, Tripeptide RGD-dependent adhesion of articular chondrocytes to synovial fibroblasts, *J. Cell Sci*. **101** (1992), 859–871.
[23] N. Rinaldi, M. Schwarz-Eywill, D. Weis, P. Leppelmann-Jansen, M. Lukoschek, U. Keilholz and T.F.E. Barth, Increased expression of integrins on fibroblast-like synoviocytes from rheumatoid arthritis in vitro correlates with enhanced binding to extracellular matrix proteins, *Ann. Rheum. Dis*. **56** (1997), 45–51.
[24] W. Schwab, A. Hofer and M. Kasper, Immunohistochemical distribution of connexin 43 in the cartilage of rats and mice, *Histochem. J*. **30** (1998), 413–419.
[25] G. Tokiwa, I. Dikinc, S. Lev and J. Schlessinger, Activation of Pyk2 by stress signals and coupling with JNK signalling pathway, *Science* **273** (1996), 792–794.
[26] M.P. Vincenti, C.I. Coon, O. Lee and C.E. Brinckerhoff, Regulation of collagenase geneexpression by IL-1β requires transcriptional and post-transcriptional mechanisms, *Nucleic Acid Res*. **22** (1994), 4818–4827.
[27] A.Z. Wang, J.C. Wang, G.W. Fisher and H. Diamond, Interleukin-1β-stimulated invasion of articular cartilage by rheumatoid synovial fibroblasts is inhibited by antibodies to specific integrin receptors and by collagenase inhibitors, *Arthritis Rheum*. **40** (1997), 1298–1307.