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Increased Intercellular Communication through Gap Junctions May Contribute to Progression of Osteoarthritis

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Our aim was to support the hypothesis of a specific association between gap junctions in synovial tissue and the presence of osteoarthritis, as evidenced by differences between osteoarthritis and non-osteoarthritis synovia in the number of gap junctions, the amount of gap-junction protein, and the amount of enzymatic activity produced through a pathway mediated by gap-junction intercellular communication. An average of 4.41 gap junctions were found per 100 cells counted in the osteoarthritis synovia, compared with 1.00 in the controls. The amount of the gap-junction protein connexin 43 in synovial lining cells was approximately 50% greater in patients with osteoarthritis. Synovial lining cells from patients with osteoarthritis produced matrix metalloproteinases constitutively and, at higher levels, in response to stimulation by interleukin-1β. In both cases, intercellular communication through gap junctions was shown to be critical to the ability of the cells to secrete matrix metalloproteinases. Overall, the results indicated that gap junctions between synovial lining cells were altered significantly in patients with osteoarthritis, as a consequence of the disease process or as part of the causal chain. In either case, gap junctions seem to be a rational therapeutic target.

Osteoarthritis (OA) and other chronic joint diseases affect almost 70 million Americans; the frequency of OA has increased significantly during the last 5 years, partly because of the aging of the population.[1] There is no cure for OA, and consequently clinical efforts aimed at treating the disease are directed toward symptomatic relief of pain. Conventional therapy for painful OA includes treatment with analgesics or nonsteroidal antiinflammatory drugs, interarticular injection of corticosteroids, viscosupplementation, and surgical procedures. Rational treatments aimed at curing OA, or at least arresting its progression, depend heavily on the development of knowledge regarding the mechanisms that mediate the disease.

Synovial lining cells are an important source of the signaling agents that regulate remodeling of joint tissues.[23,26] Dysregulation of this process leads to increased destruction of cartilage or inadequate repair, resulting in the clinical manifestations of OA.[10,26] Among the many important products of synovial lining cells are the matrix metalloproteinases (MMPs),[27] which control the breakdown of cartilage.[4] Matrix metalloproteinases are produced in response to several agents including interleukin-1β (IL-1β),[8,23] and consequently the processes that mediate the expression pathway for MMPs are potential therapeutic targets.[6]

Within a few minutes after binding to its receptor, IL-1β depolarized HIG-82 synovial fibroblasts through processes mediated by activation of protein kinase C and Ca^{2+} influx.[17] Intercellular communication through gap junctions, plaques of regulated ion channels in cell membranes that permit direct metabolic and electrical connection between adjacent cells,[7] also was necessary for the cells to respond to the cytokine.[17,18] Whether protein kinase C and Ca^{2+} influx directly regulated intercellular communication through the gap junctions is unknown, but such communication was shown to be critical to the ability of the HIG-82 cells to express MMPs in response to IL-1β.[19] Gap-junction intercellular communication occurred between synovial lining cells obtained from the knees of patients with OA.[16]

Observations that gap-junction intercellular communication mediated a characteristic biologic function of HIG-82 cells[19] and that functional gap junctions were present in

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human synovial tissue[16] led us to suspect that alterations in intercellular communication between synovial lining cells might be a cause or contributor to the progression of OA. The aim of our study was to support the hypothesis of a specific association between gap junctions in synovial tissue and the presence of OA, as evidenced by differences between osteoarthritic and nonosteoarthritic synovia in the number of gap junctions per cell, the amount of gap-junction protein per unit weight of cell protein, and the amount of enzymatic activity produced by standardized synovial biopsies through a pathway mediated by gap-junction intercellular communication.

MATERIALS AND METHODS

Surgical Procedures

Synovial biopsy specimens were obtained from patients having total knee replacement (TKR) for OA, and from patients with radiologically normal joints (control patients) having meniscectomy or reconstruction of the anterior cruciate ligament (ACL). Only patients with no radiologic evidence of OA and no arthroscopic evidence of inflammation of the synovium were accepted as control patients. All experimental procedures were approved by the Institutional Review Board for Human Research at our institution.

The tissues were taken from the suprapatellar pouch and the fat pad, using a full bite of an arthroscopic basket punch (No. 012013, Acufex, Smith & Nephew, Andover, MA); only relatively flat areas were biopsied to minimize variations in the number of synovial lining cells attributable to irregularities of the synovial surface. For the immunoblot and enzyme studies, a second bite was made as close as possible to the same location, but deeper in the tissue, to obtain tissue containing a minimal number of synovial lining cells (fat biopsy). In preliminary studies we established that the synovial lining cell and fat biopsies averaged approximately 20 mg, 0.12 cm^2, ±15%. Six biopsies of each type were obtained from each patient, unless indicated otherwise. Pertinent patient data are given in Tables 1–3.

Transmission Electron Microscopy

Immediately after the biopsy specimens were obtained, the tissues were fixed in 2.5% glutaraldehyde in 1.0 mol/L cacodylate buffer (pH 7.2) at room temperature for 24 hours. They were washed in the same cacodylate buffer, postfixed in 1% osmium tetroxide in 0.1 mol/L cacodylate at room temperature for 1 hour, washed in cacodylate buffer, and then processed for transmission electron microscopy. Each tissue was dehydrated in a graded ethanol series (10 minutes each in 50%, 70%, 95%, and two 10-minute changes in 100%), and prepared for embedding (two 10-minute changes in propylene oxide, 1 hour in 1:1 propylene-oxide/Poly/Bed 812, and then resin alone for 10 minutes). After polymerization of the resin for 16–18 hours at 70°C, sections were cut at 70 nm and observed and photographed in an electron microscope (Hitachi H-300, San Jose, CA). In a given field, a cell was considered a candidate for counting for gap junctions if it contained a nucleus or otherwise had a longest dimension of at least 5 μm. Smaller cell processes were viewed but not counted unless a gap junction was observed. A minimum of 500 cells were counted in each synovial biopsy specimen; the results for the OA and control groups were expressed in terms of the number of gap junctions per 100 cells counted and compared using the unpaired t test.

Western Blots

The tissues were frozen at −70°C and processed as a group. They were homogenized in 1 mmol/L sodium bicarbonate buffer (pH 7.4) containing 1 μg/mL leupeptin, 0.1 mmol/L tosylphenylalanine chloromethyl ketone, and 1 mmol/L phenylmethyl-sulfonyl fluoride from a fresh stock ethanol solution. The total protein concentration of the homogenates was determined using a commercial kit (Bio-Rad, Hercules, CA). The homogenates were boiled in 2× Laemmli sample buffer, and 25 μg total protein was separated by sodium dodecyl sulphate polyacrylamide gel (10% or 14%) electrophoresis. The separated proteins, which included membrane and intercellular fractions, were transferred to nitrocellulose membranes at 100 V for 1 hour. The membranes were blocked for 30 minutes at room temperature in TBST (10 mmol/L Tris-HCl, pH 8.0, 150 mmol/L NaCl, 0.5% Tween 20) containing 10% nonfat powdered milk, and then incubated for 30 minutes at room temperature with an affinity-purified polyclonal antibody against the gap-junction protein connexin 43 (Cx43) (Zymed, South San Francisco, CA) or with antibody plus peptide inhibitor at a dilution of 1 μg/mL in TBST. The membranes were washed three times for 10 minutes each in TBST and incubated with secondary antibody (antirabbit IgG[Fc]-alkaline phosphatase conjugate (Promega, Madison, WI)) at a dilution of 1:10,000

TABLE 1. Patients Studied by Electron Microscopy

Patients with Osteoarthritis			Control Patients		
Number	*Age (years)/Gender*	*Kellgren-Lawrence Grade*	*Number*	*Age/Gender*	*Surgery*
1-OA	61/F	IV	1-C	20/M	ACL Reconstruction
2-OA	58/F	IV	2-C	18/F	ACL Reconstruction
3-OA	47/F	IV	3-C	13/F	ACL Reconstruction
4-OA	63/F	IV	4-C	23/M	ACL Reconstruction
5-OA	58/F	IV	5-C	22/M	ACL Reconstruction
6-OA	53/F	IV			

ACL = anterior cruciate ligament

TABLE 2. Patients Studied by Western Blot Analysis

Patients with Osteoarthritis			Control Patients		
Number	*Age (years)/Gender*	*Kellgren-Lawrence Grade*	*Number*	*Age (years)/Gender*	*Surgery*
7-OA	68/M	IV	6-C	61/M	Meniscectomy
8-OA	68/F	IV	7-C	24/F	Meniscectomy
9-OA	60/F	IV	8-C	41/M	Meniscectomy
10-OA	67/M	IV	9-C	24/M	ACL Reconstruction
11-OA	84/F	IV	10-C	45/F	Meniscectomy

ACL = anterior cruciate ligament

for 30 minutes. After three 10-minute washes in TBST, the proteins were observed by incubating the membranes in AP buffer (0.1 mol/L Tris-HCl, pH 9.5, 0.1 mol/L NaCl, 5.0 mmol/L $MgCl_2$) containing 0.33 mg/mL nitroblue tetrazolium and 0.165 mg/mL 5-bromo-4-chloro-3-indolylphosphate. The relative intensity values of the connexin 43 bands were determined using a gel documentation system (Gel Doc; Bio-Rad), and the OA and control groups were compared using the unpaired t test.

Enzyme Assay

Immediately after the biopsy was done, the tissue sample was placed in a well in a 96-well plate containing 200 μL serumless Neuman & Tytell (NT) medium (Gibco, Carlsbad, CA). The tissues were washed four times in NT and then incubated at 37°C for 24 hours in 200 μL NT containing various combinations of human recombinant IL-1β and the gap-junction inhibitors octanol (1 mmol/L) or 18α-glycyrrhetinic acid (10 μmol/L).[5,14,28] The MMP activity of the supernatant, principally collagenase (MMP-1) and stromelysin (MMP-3),[9,21] then was measured using a film of collagen as the substrate. Matrix metalloproteinase-1 and MMP-3 are strongly associated with the presence of OA.[11,20]

For preparation of the film, collagen solution (4 μL, 3.0 mg/mL in 0.012 N HCl, Collagen Biomaterials, Palo Alto, CA) was added to each well and precipitated by slowly increasing the pH. The plate was dried at 23°C for 24 hours, washed with H_2O, and dried again at 23°C, resulting in a thin collagen film (approximately 12 μg) bound tightly to the bottom of each well.

The cell supernatants to be analyzed were filtered, and 1 mmol/L p-aminophenyl-mercuric acetate was added to activate the MMPs. Enzymatic reactions of the supernatants against the collagen films were done in triplicate at 24°C for 10 hours. The digested portion of the film was washed away, the undigested portion was fixed for 3 hours with 0.1% Coomassie Brilliant Blue G in 10% acetic acid and 45% methanol, and the wells were washed and dried. The undigested film then was dissolved in 50 μL ethanol for 20 minutes to obtain a uniform color distribution, and the optical density of each well was read with a microplate reader (MR 5000, Dynatec Laboratories, City, State) at 570 nm (wavelength of maximum Coomassie Brilliant Blue G absorbance). Additional details are given elsewhere.[19]

All reagents were obtained from Sigma (St Louis, MO) unless noted otherwise. The stock solution of IL-1β contained 0.1% bovine albumin as carrier protein. The final albumin concentration in NT did not exceed 10^{-5}%; control experiments showed

TABLE 3. Patients Studied by Metalloproteinase Assay

Patients with Osteoarthritis			Control Patients		
Number	*Age (years)/Gender*	*Kellgren-Lawrence Grade*	*Number*	*Age (years)/Gender*	*Surgery*
12-OA	80/F	IV	11-C	25/M	Meniscectomy
13-OA	70/F	IV	12-C	49/F	Meniscectomy
14-OA	61/F	IV	13-C	46/M	Meniscectomy
15-OA	76/F	IV	14-C	59/F	Meniscectomy
16-OA	69/F	IV	15-C	36/F	Meniscectomy
17-OA	55/M	III	16-C	37/M	Meniscectomy
18-OA	62/F	IV	17-C	22/M	Meniscectomy
19-OA	82/F	IV	18-C	39/F	ACL reconstruction
20-OA	69/M	IV	19-C	28/M	Meniscectomy
21-OA	69/F	IV	20-C	35/M	ACL reconstruction
22-OA	79/F	IV			
23-OA	75/F	IV			
24-OA	72/F	IV			
25-OA	55/F	IV			

ACL = anterior cruciate ligament

that 10^{-3}% bovine albumin did not influence the results of the enzyme. Octanol, 18α-glycyrrhetinic acid and p-aminophenylmercuric acetate were dissolved in dimethyl sulfoxide at a final concentration not exceeding 0.3%; control experiments showed that this concentration did not influence the measurements.

The OA and control groups were compared using the paired or unpaired t test, as indicated.

RESULTS

Morphologic evidence of the presence of gap junctions between synovial lining cells (Fig 1) was observed in all patients with OA, and in four of five control patients. The gap junctions occurred between cell processes, and only rarely involved a cell body. We determined the relative prevalence of gap junctions in OA and control patients under the assumption that a difference would support the thesis that intercellular communication had some role in the disease. On average, 4.41 gap junctions were found per 100 cells counted in the OA synovia, compared with 1.00 gap junctions per 100 cells in the controls ($p < 0.05$) (Table 4). More gap junctions probably were present; however, only a small part of each cell could be assessed

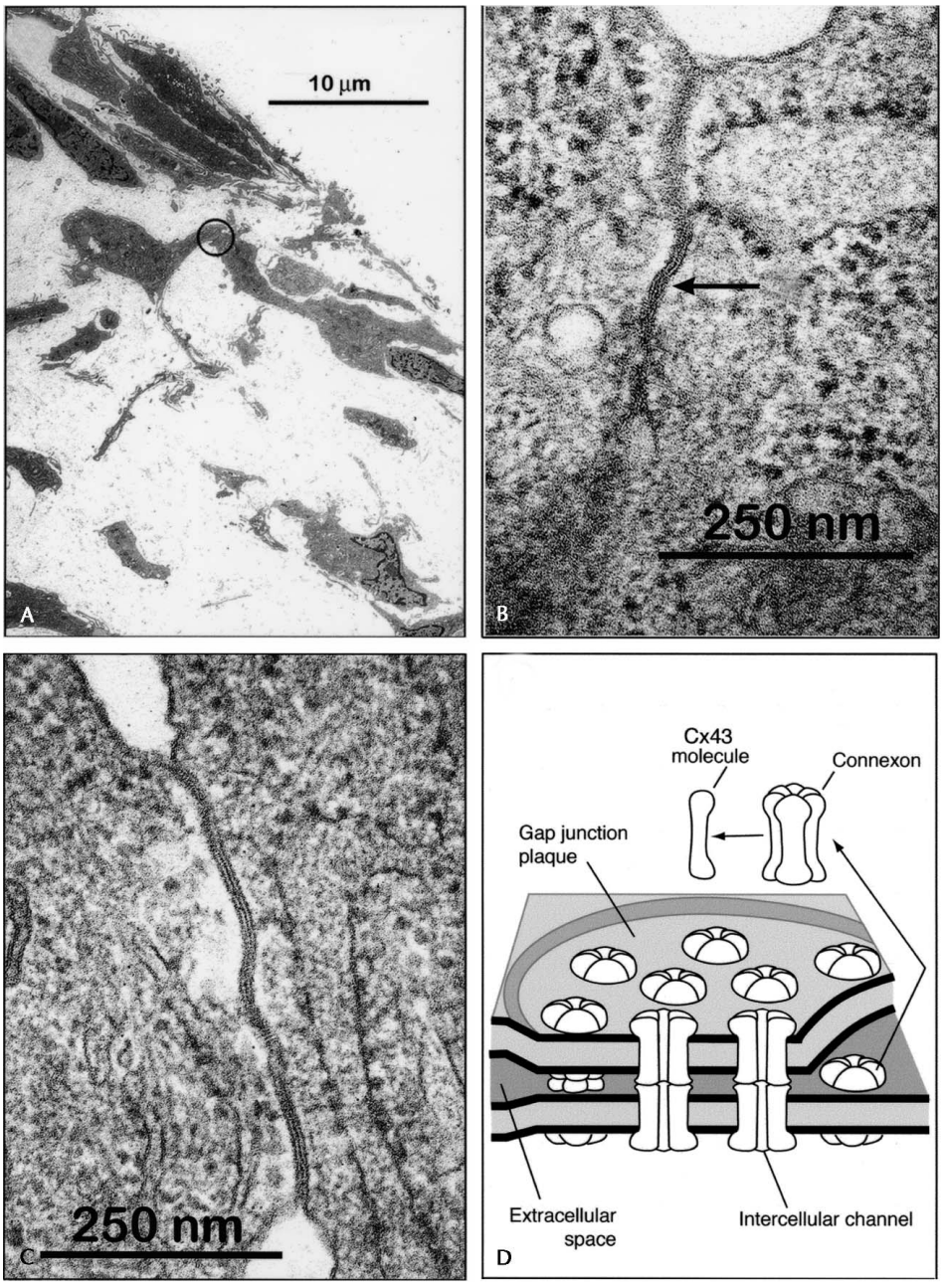


Fig 1A–D. Transmission electron micrographs of synovium from the knee of a patient with Grade IV OA show gap junctions between synovial lining cells. (A) Most cells are Type B, as evidenced by abundant rough endoplasmic reticulum (× 2500). (B) The contact area (circled in A) between two cell processes showing a gap junction is evidenced by the alternate dark and light bands along the cell membranes. The junction is best seen in the center (arrow) because of the angle of the section (× 140,000). (C) Another gap junction from the same biopsy specimen is shown (× 140,000). (D) A schematic drawing of a gap-junction plaque shows pores between two adjacent cells formed by 6 mers of Cx43 molecules (which are functional hemichannels called connexons) in the membrane of one cell aligned with connexons in the adjacent cell. For clarity, the outer membrane leaflets are shown separated (outer membrane leaflets are fused in B and C).

TABLE 4. Gap Junctions in Synovial Tissue

Patient Number	Number of Cells Counted	Number of Gap Junctions	Mean Gap Junction Length (μm)	Gap Junctions per 100 Cells
1-OA	565	23	0.44	4.07
2-OA	635	8	0.47	1.26
3-OA	526	19	0.33	3.61
4-OA	603	22	0.31	3.65
5-OA	545	40	0.35	7.34
6-OA	567	37	0.36	6.53
		Mean ± SD	0.37 ± 0.20	*4.41 ± 2.21
1-C	631	10	0.27	1.58
2-C	501	3	0.50	0.60
3-C	540	6	0.35	1.11
4-C	541	0	—	0
5-C	650	11	0.30	1.69
		Mean ± SD	0.32 ± 0.14	1.00 ± 0.71

*$p < 0.05$

OA = patients with osteoarthritis

C = control patients

because of the thinness of the tissue sections, resulting in artificially low numbers of gap junctions. The important point is that the under assessment affected both groups equally, and therefore had no relative effect. The average gap-junction length was similar in the two groups (0.37 μm and 0.32 μm in the OA and control groups, respectively) but the distributions of lengths differed between the two groups. All gap junctions in the controls were less than 0.6 μm, but in the patients with OA more than 10% of the gap junctions were greater than 0.6 μm (Fig 2).

Higher levels of connexin 43 per unit of total cell protein occurred in patients with OA (Fig 3). There are 16 mammalian connexin proteins.[2] From dual patch-clamp studies we determined that the single-channel conductance of the gap junction between HIG-82 synovial fibroblasts was approximately 70 picosiemens, suggesting connexin 43 was the gap-junction protein, as was confirmed;[3] connexin 43 exists in two phosphorylated forms and one nonphosphorylated form in the range 43–46 kDa.[22] In this study, we sought and found direct evidence that connexin 43 also was present in synovial lining cells, and that the amount of the protein differed in the presence of OA (Fig 3). Antibody against a peptide corresponding to a segment near the carboxy-terminus of connexin 43 labeled a protein within the expected range of molecular weights (43–46 kDa). For each patient, the density of the detected protein was greater in the synovial biopsy specimen compared with the fat biopsy specimen. Considering that the essential difference between the two types of biopsy specimens was the relative number of synovial lining cells, it could be concluded that the increased level of connexin 43 arose from the synovial lining cells. The antibody was specific for the peptide, as judged by the absence of staining when the antibody was incubated with the peptide before treating the blot. The mean intensity of the connexin 43 band in the patients with OA (mean difference between the S and F lanes in Figure 3) was 12.4. The corresponding value obtained from the control patients was 8.2 ($p < 0.05$), indicating that significantly more connexin 43 occurred in the synovial lining cells of the patients with OA.

Synovial lining cells obtained from patients with OA produced MMPs in response to stimulation by IL-1β (Fig 4), paralleling the results obtained previously using HIG-82 cells.[19] Most of the measured activity was produced by the synovial lining cells, as indicated by the reduced enzyme-activity levels measured in the corresponding fat biopsies. Synovial lining cells from control patients constitutively secreted MMP activity at levels only slightly above the assay baseline (Fig 5). The addition of IL-1β significantly increased the enzyme activity (Fig 5), but the resulting average level was far less than that observed from synovia of patients with OA (Fig 4).

Intercellular communication through gap junctions was critical to the ability of cells from patients with OA to produce MMPs in response to IL-1β (Fig 6). When gap-junction inhibitors were added to the culture media 10 minutes before addition of the IL-1β, an effect on IL-1β-induced MMP production occurred beginning approximately 8 hours after addition of the cytokine, and statistically significant reductions of MMP activity were found after 18 and 24 hours. The pattern of inhibition reflected in the data after they were averaged across all the patients

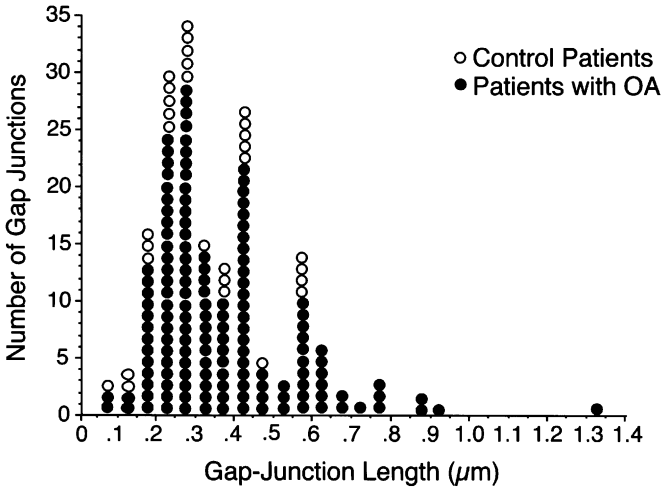
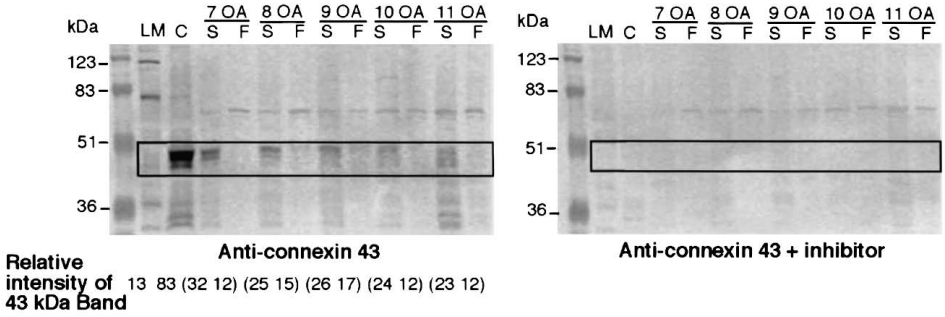


Fig 2. Each gap junction found in a patient with OA or in a control patient (Table 1) is represented by a circle and is grouped by size. All the gap junctions that were longer than 0.6 μm occurred in the patients with OA.



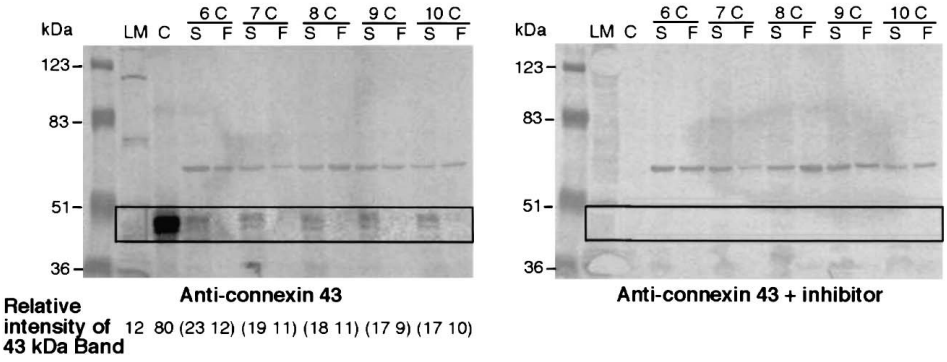


Fig 3. Immunoblots of proteins extracted from synovial and biopsy specimens show increased expression of connexin 43 in patients with OA, compared with control patients. LM, C, negative (LM cells) and positive (mouse heart) controls, respectively. The relative amount (arbitrary units) of protein in the 43 kDa band (43–46 kDa) is shown in paired synovial (S) and fat (F) biopsy specimens from five patients with OA and five control patients (Table 2).

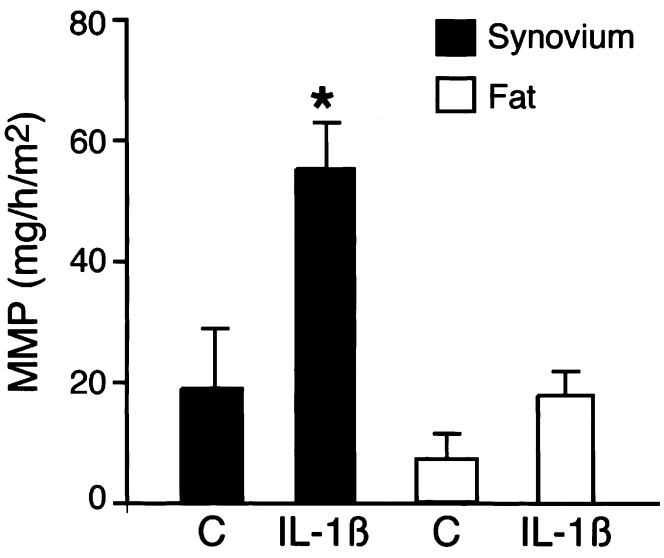


Fig 4. When synovial biopsy specimens from patients with OA were cultured in the presence of IL-1β (100 pg/mL) for 24 hours, the amount of MMP activity in the supernatant was increased, compared with control (C) synovial biopsy specimens (no IL-1β). The lower MMP values found in the fat biopsy specimens indicated that it was primarily the synovial lining cells that responded to the cytokine. (Three measurements per condition per patient; n = 6 patients, 12-OA to 17-OA; Table 3). The individual means were used to compute the overall patient means (±SE). *p < 0.05, paired t test

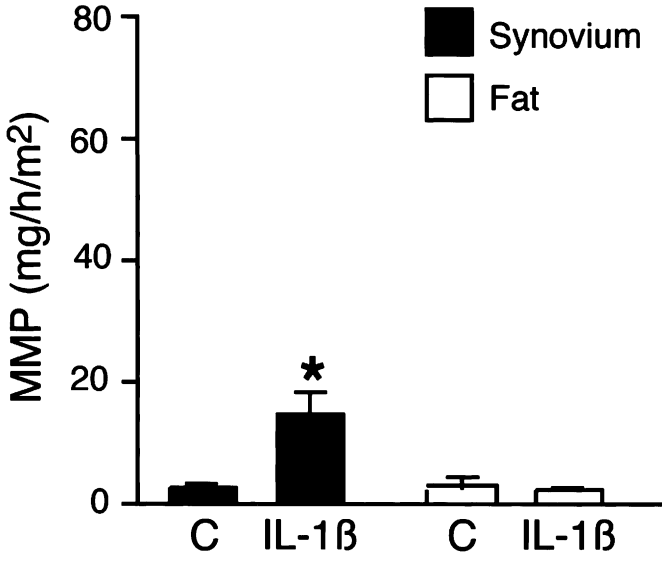


Fig 5. When synovial biopsy specimens from control patients (no OA) were cultured in the presence of IL-1β (100 pg/ml) for 24 hours, the amount of MMP activity in the supernatant was increased, compared with control (C) synovial biopsy specimens (no IL-1β). The lower MMP values found in the fat biopsy specimens indicated it was primarily the synovial cells that responded to the cytokine. (Three measurements per condition per patient; n = 10 patients; Table 3). The individual means were used to compute the overall patient means (±SE). *p < 0.05, paired t test

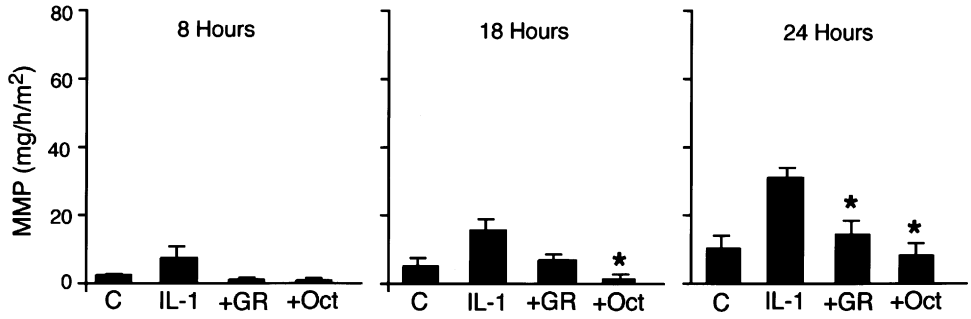


Fig 6. Gap-junction inhibitors blocked the ability of IL-1β to induce MMP production by synovial biopsy specimens from patients with OA. Control (C) (no IL-1β or inhibitors); IL-1β (100 pg/mL IL-1β); +GR, addition of IL-1β and 18α-glycyrrhetinic acid (10 μmol/L); +Oct, addition of IL-1β and octanol (1 mmol/L). Octanol or 18α-glycyrrhetinic acid were added to the culture media 10 minutes before addition of IL-18. Matrix metalloproteinase activity was measured after the tissues were exposed in culture to the indicated conditions for 8, 18, and 24 hours. (Three measurements per condition per patient; n = 4 patients, 15-OA, 18-OA, 19-OA, 20-OA; Table 3; Mean ± SE, *, $p < 0.05$, compared with IL-1β, paired t test)

(Fig 6) also occurred at each time studied in each of four patients (data not shown).

The constitutive secretion of MMP activity by OA synovia (in patients in which it occurred [see below]) was also mediated by gap-junction intercellular communication (Fig 7). Each gap-junction inhibitor significantly reduced the amount of MMP activity secreted into the culture media.

DISCUSSION

The ability of cells to communicate directly through gap junctions, which probably occur between most cell types including tendon cells,[24] chondrocytes,[25] osteoblasts,[29] meniscus cells,[12] and synovial lining cells,[16] has led to the idea that gap junctions transmit metabolic regulatory signals, and there now is a need to define the circumstances in which the intercellular communication actually determines the overall cellular response. If progression of OA were one such case, we would expect that intercellular communication would be critical to secretory pathways leading to clinical manifestations of the disease. We also would expect specific differences in measures of gap junctions between the presence and absence of OA. To test these hypotheses we did morphologic and functional studies in which we compared synovial biopsy specimens from patients with Grade IV OA and patients who showed no clinical signs of the disease (controls). The average age of the patients with OA differed significantly from the control group (66 versus 33 years) (Tables 1–3). We did not find any evidence, either in the literature or in this study, suggesting that a patient's age could affect the parameters evaluated. Nevertheless, the possibility exists.

On average, there were significantly more gap junctions per cell between synovial lining cells in synovia of patients with OA (Table 4). Gap junctions are planar structures having irregular areas[15] that appear as linear segments of plasma membrane when viewed transversely in represen-

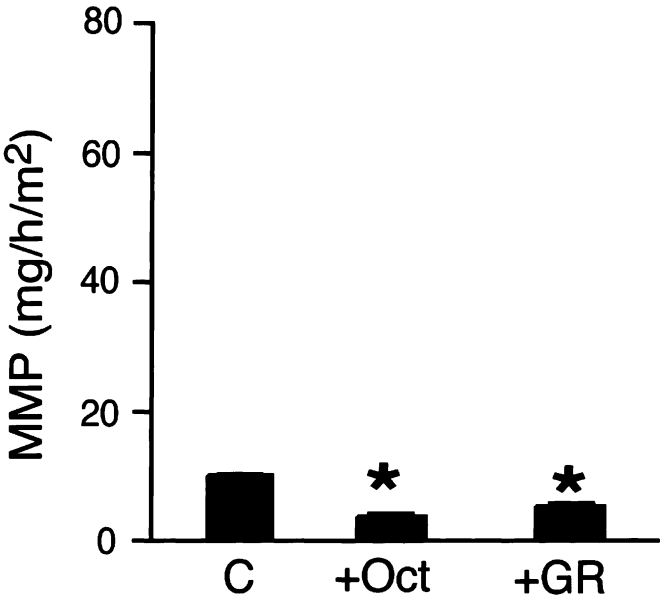


Fig 7. Gap-junction inhibitors significantly reduced the amount of constitutive secretion of MMPs by synovial biopsy specimens from patients with OA. Control (C) (no gap-junction inhibitors); +GR, addition of 18α-glycyrrhetinic acid (10 μmol/L); +Oct, addition of octanol (1 mmol/L). The tissues were exposed in the presence and absence of the inhibitors for 24 hours, and the enzymatic activity of the supernatant was measured. (Three measurements per condition per patient, n = 5 patients, 21-OA to 25-OA, Table 3; Mean ± SE, *, $p < 0.05$, paired t test)

tative tissue sections. The measured segment lengths only rarely reflected the area of the gap junctions. Nevertheless, because the sections were cut randomly, our observation that large gap junctions (> 0.6 μm) occurred only in OA synovia suggests that the gap junctions that occurred there were larger. The number of synovial lining cells in each biopsy specimen was not quantitated, but we estimate it was greater in the OA group by a factor between 1 and 2. Therefore, on the basis of morphologic evidence, OA synovia contained more gap junctions and a greater absolute number of gap junctions, and likely contained more large gap junctions.

Synovial lining cells are divided into Type A cells, principally of the macrophage lineage, and Type B or secretory cells.[13] It generally was not possible to determine whether the gap junctions were of the A-A, B-B, or A-B types because the junctions almost always occurred between cell processes, and the cell bodies (that must be present to allow a determination of cell type) usually were not present in the same field as the gap junction.

Approximately 50% more connexin 43 per unit of extracted protein was found in the biopsy specimens from patients with OA (Fig 3), suggesting an association between gap junctions and the presence of OA. We did not address questions involving the level at which the regulation of connexin 43 was altered or the possible involvement of another connexin protein in the formation of the gap junctions. The open state of connexin 43 can be regulated by phosphorylation, and there is a 3 kDa difference in the molecular weight of the phosphorylated and unphosphorylated forms.[22] Our blots were not designed to resolve this difference, and consequently it is questionable whether the presence of OA is associated with a characteristic change in the extent of phosphorylation of connexin 43. Nevertheless, the phosphorylated forms of connexin 43 were predominant in both groups of patients, as can be seen in the bold upper band in lane C (positive control) around 43 kDa, and in the lanes of the synovial lining cells. Despite the unresolved questions regarding the exact role of connexin 43 in mediating OA, it is clear that an increased amount of the protein was present in association with the disease, thereby adding support to the hypothesis that the function mediated by the protein had a specific relationship to the disease.

Synovial lining cells in culture expressed MMPs constitutively and in response to stimulation with IL-1β; MMP expression was greater in biopsies from patients with OA (Fig 4), compared with those from control patients (Fig 5). For patients with OA, intercellular communication through gap junctions between synovial lining cells was essential for stimulated (Fig 6) and constitutive (Fig 7) secretion of MMPs as evidenced by the significant reductions of MMP activity after the cells were cultured for 24 hours in the presence of the gap-junction inhibitors. When the synovial lining cells were stimulated with IL-1β, the response pattern to the inhibitors reflected in the data after they were averaged across the patients (Fig 6) also occurred in each patient studied (data not shown). The average effect of the inhibitors on constitutive secretion (Fig 7) was similarly reflected in the measurements from each patient (data not shown). Because interpatient variation was small, resulting in consistent patterns from patient to patient, it can be inferred that intercellular communication was necessary in each patient rather than only at a general or average level. The possibility cannot be excluded that both effects (Figs 6, 7) were nonspecific and did not evidence a role for intercellular communication in the secretory activity of synovial lining cells. However, that possibility seems unlikely because it would require two standard, chemically distinct gap-junction inhibitors[5,14,28] to produce a nonspecific effect of approximately the same amount.

Overall, the structural, biochemical, and functional lines of inquiry yielded results supporting the view that intercellular communication between synovial lining cells was significantly altered in patients with OA, compared with patients who did not manifest the disease. The changes in intercellular communication could have been a consequence of the disease process or part of the causal chain. In either case, because intercellular communication was essential for cell expression of MMPs, overexpression of which results in the clinical manifestations of OA, gap junctions seem to be a rational therapeutic target. Potentially useful agents include those that directly inhibit gap-junction intercellular communication, and agents that inhibit earlier stages in the signal transduction process including Ca^{2+} influx and protein kinase C-gated Na^{+} channels.[17,18]

A comparison of the MMP response between patients with and without OA revealed that the synovial lining cells were phenotypically different in the context of the disease (Fig 8). Stimulated MMP activity in the OA group was more than a factor of three greater ($p < 0.05$) than in the controls, suggesting that synovial lining cells of patients with OA were abnormally able to be activated, because not all the increased activity could be accounted for by an increased density of synovial lining cells (which increased only by a factor of 1 or 2 in the patients with OA). Moreover, the average amount of MMP activity secreted constitutively by the cells from the patients with OA was 18.7 ± 10.2 mg/hour/m^2, whereas the corresponding average in control patients barely was above the assay baseline (Fig 8). This difference was not statistically significant because of the large standard error in the patients with OA. However, in three of the patients (12-OA, 13-OA, 14-OA) the cells in the synovial biopsy specimen did not constitutively

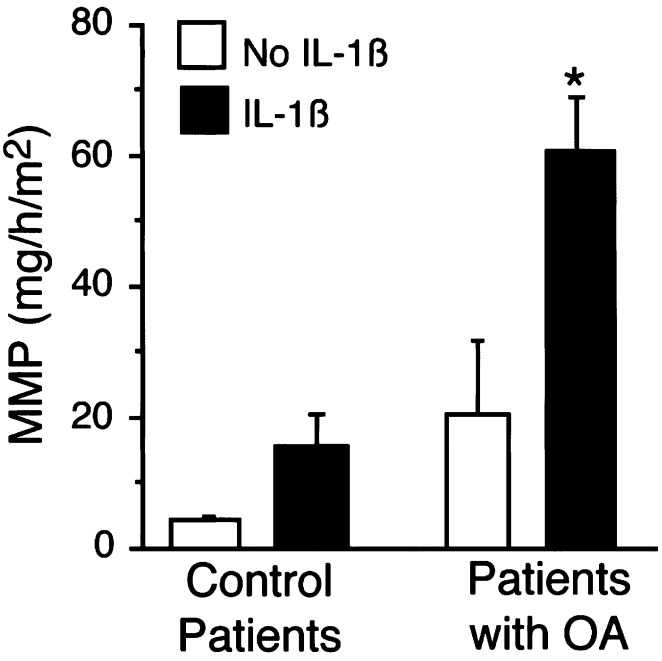


Fig 8. Synovial biopsy specimens from patients with OA produced significantly more IL-1β-induced MMP activity, compared with patients who did not have OA. Data from Figures 4 and 5. Mean ± SE, *, $p < 0.05$ compared with control patients, unpaired t test

secrete any MMP activity, and in three others (15-OA, 16-OA, 17-OA) the cells constitutively produced 17–58 mg/hour/m^2. Therefore, there might have been two subgroups among the patients with OA regarding constitutive expression of MMP activity, which would be significant phenotypic alteration compared with the control patients. The possibility cannot be resolved on the basis of the current data, but it emphasizes the desirability of blocking on individual patients when evaluating functional end points of synovial lining cells to avoid the possibility of averaging away real differences.

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