Ex Vivo Soft-Laser Treatment Inhibits the Synovial Expression of Vimentin and α-Enolase, Potential Autoantigens in Rheumatoid Arthritis

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Background. Soft-laser therapy has been used to treat rheumatic diseases for decades. The major effects of laser treatment may be dependent not on thermal mechanisms but rather on cellular, photochemical mechanisms. However, the exact cellular and molecular mechanisms of action have not been elucidated.

Objective. The aim of this study was to investigate the *ex vivo* effects of low-level laser treatment (with physical parameters similar to those applied previously) on protein expression in the synovial membrane in rheumatoid arthritis (RA).

Design. Synovial tissues were laser irradiated, and protein expression was analyzed.

Methods. Synovial membrane samples obtained from 5 people who had RA and were undergoing knee surgery were irradiated with a near-infrared diode laser at a dose of 25 J/cm^2 (a dose used in clinical practice). Untreated synovial membrane samples obtained from the same people served as controls. Synovial protein expression was assessed with 2-dimensional polyacrylamide gel electrophoresis followed by mass spectrometry.

Results. The expression of 12 proteins after laser irradiation was different from that in untreated controls. Laser treatment resulted in the decreased expression of α-enolase in 2 samples and of vimentin and precursors of haptoglobin and complement component 3 in 4 samples. The expression of other proteins, including 70-kDa heat shock protein, 96-kDa heat shock protein, lumican, osteoglycin, and ferritin, increased after laser therapy.

Limitations. The relatively small sample size was a limitation of the study.

Conclusions. Laser irradiation (with physical parameters similar to those used previously) resulted in decreases in both α-enolase and vimentin expression in the synovial membrane in RA. Both proteins have been considered to be important autoantigens that are readily citrullinated and drive autoimmunity in RA. Other proteins that are expressed differently also may be implicated in the pathogenesis of RA. Our results raise the possibility that low-level laser treatment of joints affected with RA may be effective, at least in part, by suppressing the expression of autoantigens. Further studies are needed.

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Low-level laser therapy (LLLT) is the application of a low-power (1- to 500-mW) laser to promote tissue regeneration, reduce inflammation, and relieve pain. The wavelength typically used is in the red or near-infrared spectrum (600–1,000 nm). The irradiance applied is between 0.001 and 5 W/cm^2.[1] Low-level laser therapy has been used for almost 30 years to manage rheumatoid arthritis (RA), osteoarthritis (OA), and other rheumatic conditions.[1–4] Low-level laser therapy is considered to have moderately favorable effects on clinical symptoms[2–9] and quality of life in people with RA.[10] Inefficacy also has been reported.[11,12] In a Cochrane Database Systematic Review,[13] randomized, placebo-controlled trials of LLLT in patients with RA were analyzed. The 5 trials included in the final analysis involved 222 patients; 130 of those patients were randomized to receive laser treatment. Compared with the control, LLLT significantly reduced pain and duration of morning stiffness and increased fingertip-to-palm distance. There were no differences in other outcomes, such as functional assessment, range of motion, and local swelling, between the groups. There were no significant differences between subgroups in terms of LLLT dosage, wavelength, site of application, or treatment length. The authors concluded that LLLT could be considered for short-term treatment for the relief of pain and morning stiffness in patients with RA. They clearly stated the need to investigate the effects of the above-mentioned factors on the efficacy of LLLT for RA in randomized controlled trials.

The mode of action of LLLT has been attributed to its thermal effects.[2,4,14] However, the possibility that LLLT acts via photochemical mechanisms has been raised.[1] Today photochemical effects seem to be the major mechanism of LLLT action. Thermal effects are irrelevant. There have been scattered reports of the possible cellular and molecular anti-inflammatory effects of LLLT in RA. These effects include the suppression of tumor necrosis factor α, interleukin 1, and interleukin 8 protein and messenger RNA expression[15,16] and decreased circulating immunocomplex levels.[2] However, there have been no reports on the effects of laser irradiation on the synovial expression of citrullinated proteins.

The expression of citrullinated proteins in the synovial membrane in RA, but not in the normal synovial membrane, has been proven.[17] *In vivo*, these proteins induce the production of anti–citrullinated protein antibodies, such as anti–mutated vimentin and anti–α-enolase antibodies.[18,19] In our placebo-controlled, prospective investigations, phosphate-glass laser irradiation of rheumatoid joints resulted in significant decreases in synovitis and acute-phase reactant levels compared with the results observed in people treated with placebo.[20]

The short-term efficacy of LLLT in RA is evidence based.[13] Molecular anti-inflammatory effects of LLLT have been reported in a few studies.[2,15,16] In our previous study of *ex vivo* laser irradiation of rheumatoid synovial tissues, we found dose-dependent changes in the synthesis of synovial proteins.[3] At that time, there was no suitable method for the identification of those proteins. Recently, synovial proteomic analysis became a useful tool in arthritis research.[21,22] Mass spectrometry identified autoantigens in RA.[23]

In the present study, we wanted to investigate the effects of *ex vivo* laser irradiation (with parameters similar to those used in our clinical practice) of rheumatoid synovial tissues on protein expression,

Table 1.
Characteristics of the 5 Participants With Rheumatoid Arthritis (RA)[a]

Participant No.	Age (y)	Duration of RA (y)	DAS28 (at Start of Study)	Ongoing Corticosteroid Therapy	Ongoing DMARD Therapy	C-Reactive Protein Level (mg/L)	Erythrocyte Sedimentation Rate (mm/h)	Anti-CCP Antibodies[b] (IU/mL)	Anti-MCV Antibodies (IU/mL)
1	63	30	4.28	Yes	Yes		7	43.7	52.9
2	67	23	5.13	Yes	Yes	13.5	45	2,328.1	804.1
3	34	20	5.06	Yes	Yes	17.0	74	1.0	11.2
4	59	7	5.17	No	Yes	16.9	14	10.0	4.7
5	54	28	4.21	No	Yes	7.0	56	3.0	26.3

[a] DAS28=28-joint-count disease activity score, DMARD=disease-modifying antirheumatic drug, CCP=cyclic citrullinated peptide, MCV=mutated citrullinated vimentin.
[b] Determined with a second-generation anti-CCP assay.

including that of the recently discovered possible autoantigens. Arthroscopy, including laser surgery, is frequently used in the treatment of rheumatoid joints.[24] Intra-articular LLLT of rheumatoid knees also may be a future therapy. Therefore, we aimed to study the effects of LLLT on rheumatoid synovial tissue samples obtained from synovial biopsies.

Method

Participants

Five women fulfilling the American Rheumatism Association criteria for the classification of RA[25] underwent total knee replacement surgery because of progressive, destructive disease. The demographic, clinical, and laboratory characteristics of the women are shown in Table 1. All participants signed an informed consent form.

Laser Irradiation

Laser irradiation was performed with the same KLS-500 near-infrared diode laser* that we use for the percutaneous treatment of rheumatoid knee joints. Laser specifications were as follows: wavelength, 809 nm; power, 448 mW; output aperture size, 2 × 4.5 mm; beam divergence, 5 degrees. In our clinical practice, the knee joint is treated with this laser at 16 to 20 different points and a dose of 4 J per point. The treatment course includes 10 to 15 sessions, for a total dose of 40 to 60 J. The laser output aperture area (2 × 4.5 mm) provides a radiation exposure of the dermal surface of 444 to 666 J/cm^2. For depth of penetration into the skin and connective tissues, 1- and 3-mm values were found in the literature, respectively.[26,27] The energy of laser radiation decreases to 37% of the incident energy as it passes through the tissue if its thickness is equal to the penetration depth. Assuming a 2-mm dermal width and a 3-mm width for adipose and connective tissues, the radiation exposures reaching the synovial membrane were calculated to be $0.37^3 \times 444 = 22.5$ and $0.37^3 \times 666 = 33.3$ J/cm^2, respectively.

For the *in vitro* irradiation of synovial membrane samples, an approximate mean radiation exposure of 25 J/cm^2 was chosen to model real exposure during treatment. One of the 2 tissue samples obtained from the same participant was irradiated with the laser at 8 cm from the aperture for 47 seconds; the corresponding control specimen was kept under similar conditions but left untreated. In this experiment, the thermal effects of laser treatment were negligible.

Sample Preparation and Protein Extraction

Surgically removed synovial tissue samples from knees affected by RA were stored in Medium 199 (product no. M4530)† at 4°C until use. After the removal of excess adipose and connective tissues, two 0.5-cm^2 samples per participant, one for laser treatment and one to serve as a control, were cut from 2 adjacent areas. After irradiation, the samples were incubated in Medium 199 (21 hours, 37°C, 5% CO_2, Heto-Holten incubator‡) and then stored at −70°C until use. Frozen samples were prepared as described in the Ettan DIGE System User Manual[28] with minor modifications. In brief, the samples were homogenized on ice (IKA-Werk Ultra Turrax§) in 300 μL of lysis buffer (pH 8.5),† which was composed of 7M urea, 2M thiourea, 30mM tris(hydroxymethyl)aminomethane (Tris), and 4% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS). Homogenates were sonicated for 30

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§ IKA-Werk GmbH & Co KG, Janke & Kunkel Strasse 10, D-79219, Staufen, Germany.

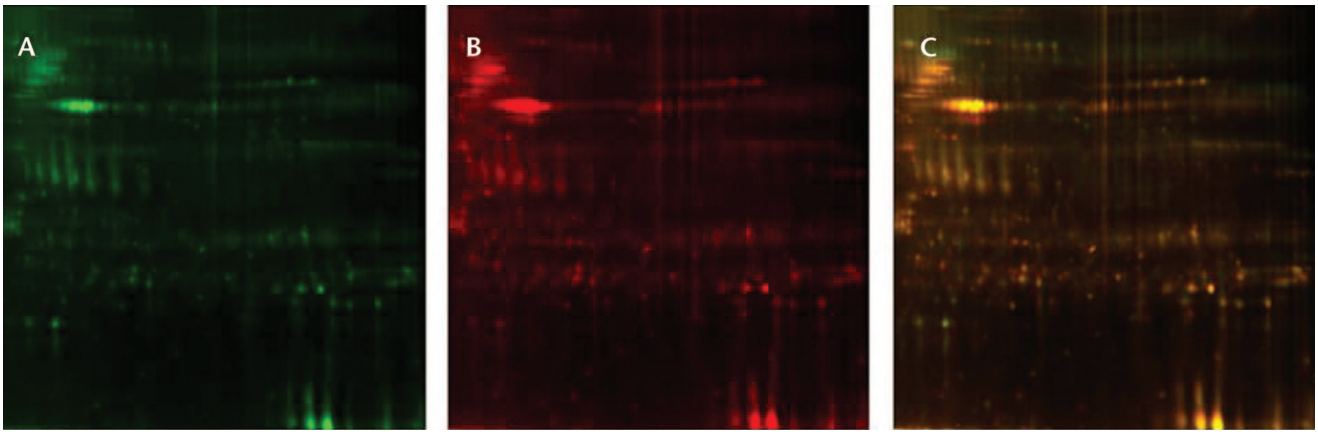

Figure.
Images of a representative gel labeled with Cy3 (A) and Cy5 (B) and the overlap of the 2 images (C).

seconds (Sonorex TK52[‖]) and centrifuged for 1 hour at 12,000*g* and 4°C. Proteins in the supernatants were precipitated with acetone (1:4, vol/vol) and centrifuged for 10 minutes at 3,000*g* and 4°C. Protein samples were stored in lysis buffer at −20°C until further analysis.

Protein Separation With 2-Dimensional Differential Gel Electrophoresis (DIGE)

Protein concentrations in the solutions were determined with a 2D-Quant Kit[#] by spectrophotometry at 480 nm according to the manufacturer's instructions.[29] Twenty-five milligrams of protein from each sample were added to the internal standard; all other samples contained 50 μg of protein. Control and laser-treated samples were labeled with Cy3 and Cy5, and the internal standard was labeled with Cy2 CyDye DIGE Fluor minimal fluorescent dyes.[#] Lysine and buffer (8M urea, 4% CHAPS, 15% glycerol, 13 mM dithiothreitol [2 mg/mL], and 0.5% IPG buffer [5 μL/mL]) were added to increase the volume to 450 μL. Strips (pH 3–10) that were 24 cm long were used during rehydration (24 hours in the dark).

Isoelectric focusing was performed for 23.5 hours at 20°C in the dark with a Multiphore II device.[#] The following sequences were applied: hold (300 V; 5.5 hours), gradient 1 (600 V; 7 hours), gradient 2 (1,000 V; 3 hours), gradient 3 (8,000 V; 3 hours), and step by hold (8,000 V; 5 hours). Next, sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed for 45 minutes at 20°C and 2 W per gel and then for 5 to 7 hours at 20°C and 12 W per gel.

Gels were scanned with a Typhoon TRIO+ device.[#] The Figure shows 3 images of a representative gel labeled with Cy3 and Cy5 and the overlap of the 2 images.

Scanned images were analyzed with DeCyder Differential Image Analysis and DeCyder Biological Variation Analysis software.[#] Image analysis can identify protein spots that are differentially expressed in laser-irradiated versus untreated synovial tissues.

Preparation of Preparative Gel for Protein Identification

For protein identification, preparative gels were made. The protocol used for preparative gels was similar to that used for analytical gels, except that 800 μg of protein was applied to gel strips and no staining was performed. Isoelectric focusing was performed according to the protocol described above. After electrophoresis, gels were fixed for 1 hour (20% methanol and 1% phosphoric acid) and stained with methanol-A-B solution (2:8:1) for 12 hours (A: 0.3M ammonium sulfate and 2.4% phosphoric acid; B: 5% Coomassie Blue G-250[**]). Gels were then neutralized for 3 minutes in 0.05M Tris solution (pH 6.5), washed in 25% methanol, and stabilized in 0.75M ammonium sulfate for 8 hours.

Protein Identification

Because Coomassie Blue G-250 staining is not as sensitive as fluorescent CyDye labeling, fewer protein spots were detectable on preparative gels than on analytical gels. Thus, only 12 protein spots were cut from the preparative gels. Proteins were identified by liquid chromatography coupled to tandem mass spectrometry

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[**] Thermo Scientific, Pierce Biotechnology Inc, 3797 N Meridian Rd, Rockford, IL 61105.

Table 2.
Quantitative Changes in Synovial Protein Expression in Rheumatoid Arthritis After Laser Irradiation[a]

Spot Identification No.	Proteins Identified by 2 or More Peptides	Proteins Identified by 1 Peptide	Participants[b]	*P* (Student *t* Test)	Mean Ratio[c]
48	96-kDa heat shock protein		1 and 3	.046	2.21
100	Lumican precursor		3 and 5	.038	1.28
321	α-Enolase		3 and 2	.024	−1.31
418	Vimentin and haptoglobin precursor (isoform CRA_b)	Complement component 3 precursor	1, 2, 3, 4, and 5	.031	−1.47
500	CGI-150 protein and osteoglycin (preproprotein isoform 1)	Guanine nucleotide–binding protein (β4 subunit)	1, 2, 3, 4, and 5	.025	2.76
426		70-kDa heat shock protein	3 and 5	.035	1.17
645		Ferritin (light polypeptide variant)	1 and 3	.038	7.07
750		Hypothetical protein LOC51237	2 and 3	.047	1.50

[a] Relative to that in untreated synovial samples. Only samples showing significant changes in protein expression ($P<.05$) are included.
[b] Only participants for whom the software found proteins on the gel are included.
[c] Ratio of after laser treatment/before laser treatment (extent of change in synovial protein expression).

at the University of Szeged, Szeged, Hungary, as described earlier.[30] After digestion of the gel samples in 0.1M ammonium-hydrocarbonate buffer and trypsin[††] for 16 hours, 2 μL of a sample were injected for liquid chromatography coupled to tandem mass spectrometry. Mass spectrometric data were analyzed by use of a SpectrumMill[‡‡] in-house server with the National Center for Biotechnology Information Homo Sapiens database. During the search, a precision of 1.6 Da was applied for precursor ions, and a precision of 0.6 Da was applied for fragments. The identification of proteins was accepted when the peptide matching score was greater than 10 and the total protein matching score was greater than 20.

Levels of Anti–Citrullinated Protein Antibodies in Serum

Anti-cyclic citrullinated peptide (CCP) immunoglobulin G levels were determined with a second-generation anti-CCP enzyme-linked immunosorbent assay (ELISA) (QUANTA Lite CCP ELISA[§§]). The test was performed in accordance with the manufacturer's instructions, and values above 20 IU/mL were considered positive.[31]

Anti-mutated citrullinated vimentin (MCV) immunoglobulin G levels were assessed with an ELISA.[‖‖] This assay contains recombinant MCV as an antigen. The test was performed in accordance with the manufacturer's instructions. The cutoff value for anti-MCV antibodies was 20 U/mL.[32]

Statistical Analysis

For DIGE experiments, an analysis of variance was calculated with DeCyder Biological Variance Analysis software. *P* values were determined for protein spots with the Student *t* test (Tab. 2), and differences were considered statistically significant when *P* values were $<.05$. The internal standard was a pool of equal amounts of all samples (treated and control samples) in the experiment. The standard provided an average image against which all other gel images were normalized, thus removing much of the experimental variation and reducing the gel-to-gel variation. Determination of the relative abundance of the fluorescent signal for the internal standards across all gels, therefore, provided standardization for all gels.

Role of the Funding Source

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Results

In the present study, changes in protein expression in 5 synovial membrane samples from participants with RA were studied after *ex vivo* LLLT irradiation. We identified 12 proteins that were expressed differently in laser-irradiated synovial tissue samples and untreated control samples. The main characteristics of these proteins are shown in Table 3. Protein match scores indicated the precision of mass spectrometric identification; the proteins that were most convincingly identified, in order of decreasing match scores, were vimentin, lumican precursor, 96-kDa heat shock protein (HSP96), CGI-150 protein, α-enolase,

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[‡‡] Agilent Technologies, 5301 Stevens Creek Blvd, Santa Clara, CA 95051.
[§§] INOVA Diagnostics Inc, 9900 Old Grove Rd, San Diego, CA 92131.
[‖‖] OrgenTec Diagnostika GmbH, Carl-Zeiss-Strasse 49, D-55129, Mainz, Germany.

Table 3.
Characteristics of Differentially Expressed Synovial Proteins

Category	Spot Identification No.	Protein Match Score	Molecular Mass (kDa)	Isoelectric Point	Identified Protein
Proteins identified by 2 or more peptides	48	80.3	92,469	4.76	96-kDa heat shock protein (gp96; tumor rejection antigen 1)
	100	84.3	38,429	6.16	Lumican precursor
	321	37.5	47,197	7.01	α-Enolase
	418	116.6	53,652	5.06	Vimentin
		33.7	50,679	6.44	Haptoglobin precursor (isoform CRA_b)
	500	48.6	55,012	8.99	CGI-150 protein
		28.3	36,096	6.34	Osteoglycin (preproprotein isoform 1)
Proteins identified by 1 peptide	418	15.1	187,149	6.02	Complement component 3 precursor
	426	14.8	72,333	5.07	70-kDa heat shock protein
	500	15.2	37,567	5.59	Guanine nucleotide–binding protein (β4 subunit)
	645	13.9	26,826	5.53	Ferritin (light polypeptide variant)
	750	17.3	20,696	5.37	Hypothetical protein LOC51237

haptoglobin precursor (isoform CRA_b), and osteoglycin (preproprotein isoform 1). It is currently believed that the most important proteins involved in articular diseases are vimentin, α-enolase, and osteoglycin.

Table 2 shows the quantitative changes in protein expression after laser irradiation. Laser treatment resulted in significantly decreased expression of α-enolase in 2 of 5 samples and of vimentin and precursors of haptoglobin and complement component 3 in 4 of 5 samples ($P<.05$). Other proteins, including lumican precursor, HSP96, CGI-150 protein, osteoglycin, and some others, showed significantly increased expression after laser irradiation relative to that in untreated tissue samples ($P<.05$).

Discussion

Numerous reports, including meta-analyses and a Cochrane systematic review, found moderate clinical efficacy of LLLT, particularly short-term beneficial effects on pain and morning articular stiffness[2,4,5,7,8,11,13–16]; however, a few investigators reported no effect at all.[9,11] Some authors reported that LLLT may even improve the quality of life of patients with RA.[10] Little information is available on the possible cellular and molecular anti-inflammatory effects of laser irradiation of the rheumatoid synovium. There are a few reports on the inhibitory effects of LLLT on synovial cytokine and immunocomplex production.[2,15,16]

As shown in Table 4, proteomic analysis of rheumatoid synovial cells, tissue, and fluid was performed by various groups.[21,33–39] Lorenz et al[40] performed proteome analysis of diseased joints of mice with collagen-induced arthritis. In the present study, only proteins that were expressed differently after laser irradiation of rheumatoid synovial tissues were identified. Among the proteins showing significantly decreased expression in laser-irradiated rheumatoid synovial tissues, α-enolase and vimentin play important roles in the pathogenesis of RA.[32,41–44] α-Enolase, a glycolytic enzyme that is involved in phosphopyruvate synthesis, also has been detected in the rheumatoid synovial membrane.[42] The immunologically dominant antigenic epitope is citrullinated α-enolase peptide 1.[19,42] An autoantibody against this peptide has been detected in the sera of 37% to 62% of patients with RA but in the sera of only 2% to 3% of patients with seronegative arthritis or people who are healthy.[19,42,45] The diagnostic specificity of α-enolase in early RA is 97%.[45]

The other RA-specific autoantibody, that against citrullinated vimentin, was first identified in 1994 and named anti-Sa (after a patient).[46] It was later demonstrated that anti-Sa specifically recognizes citrullinated vimentin.[18] Vimentin is an intermediary filament secreted and citrullinated by macrophages, mesenchymal cells, and fibroblastlike synoviocytes in response to apoptosis or by proinflammatory cytokines, such as tumor necrosis factor α.[47] Citrullinated as well as mutated vimentin is probably an autoantigen in RA.[18,48] In the present study, we used an anti-MCV ELISA to detect

this antibody in the sera of people with RA. The anti-MCV assay showed 9% higher sensitivity than the second-generation anti-CCP assay and 4% higher sensitivity than an anti–rheumatoid factor immunoglobulin M assay.[32] Both anti-Sa and anti-MCV antibodies are good prognostic predictors of structural damage in RA.[49,50]

There have been some attempts to use proteomic analysis for the detection of citrullinated proteins in the synovium and synovial fluids,[38,51–53] but there are no published data regarding the possible effects of LLLT on citrullinated synovial antigens. In the present study, laser irradiation diminished the expression of these proteins in rheumatoid explants.

Among the other, noncitrullinated proteins showing decreased expression in laser-irradiated synovial samples, haptoglobin is an acute-phase reactant that undergoes glycosylation under inflammatory conditions.[54] Increased haptoglobin production has been found in RA; a significant correlation has been found between serum haptoglobin levels and clinical activity measured with the 28-joint-count disease activity score.[55] Mass spectrometric proteome analysis of rheumatoid sera and synovial fluids revealed that this protein is expressed differently in RA than in OA or reactive arthritis.[34]

Complement component 3 is a central constituent of the complement cascade. Complement component 2 and factor B activate complement component 3, the major opsonin of this system. Complement component 3 binds to its receptors on phagocytic cells.[56] Complement component 3 has been detected in the synovium in the presence of both RA and OA.[57] Although LLLT has been shown to suppress immunocomplex levels in RA,[2] there have been no reports on the effects of LLLT on synovial complement factors.

Table 4.
Review of Proteins Differentially Expressed in the Rheumatoid Synovium

Study	Synovial Tissue	Synovial Fibroblasts	Synovial Fluid
Böhm et al[77]	Metargidin		
Dasuri et al[21]		HC gp96	
		BiP	
		Galectin 1	
		Galectin 3	
Drynda et al[33]			Calgranulin B (MRP14)
Kim et al[36]	Tropomyosin		
	Adipocyte-binding protein		
	Peroxiredoxin 2		
	Galectin 1		
	Apo-a1		
Lorenz et al[37,40]	Stat1		
	p47phox		
	Mn superoxide dismutase		
Matsuo et al[38]	Asporin		
	F-actin capping protein α-1		
Robert-Pachot et al[78]	Carbonic anhydrase III		
Sinz et al[34]			Calgranulin B (MRP14)
			Serum amyloid A protein
Tilleman et al[52]	Calgranulin A		
Uchida et al[39]			Myeloid-related protein 8
Weiler et al[79]	c19orf10	c19orf10	c19orf10

Among proteins showing increased expression in laser-irradiated samples, lumican is a corneal keratan sulfate proteoglycan and a small leucine-rich repeat protein. It also has been detected in rheumatoid synovial tissues.[58,59] After the removal of keratan sulfate residues, macrophages readily stick to the lumican core protein. Lumican may localize inflammatory macrophages at certain sites within the rheumatoid synovium.[59]

Fibromodulin is structurally related to lumican. It binds to type I and II collagen and may be implicated in collagen fibril organization. Fibromodulin and lumican bind to the same region within collagen.[60,61] Fibromodulin-deficient mice have abnormally thin type I collagen fibrils.[62] These mice eventually develop arthritis.[63]

Osteoglycin, also known as mimecan or osteoinductive factor, is a glycoprotein that induces ectopic bone formation. Osteoglycin is a natural inhibitor of osteoclast hyperfunction.[64] Hamajima et al[65] reported that LLLT stimulated the expression of the osteoglycin gene in cultured osteoblasts. We showed for the first time that laser irradiation increased osteoglycin expression in rheumatoid synovial tissue samples.

The 70-kDa heat shock protein (HSP70) is a stress-induced intra-

cellular chaperone that prevents protein aggregation. Upon cellular stress, trauma, or inflammation, HSP70 is released by affected cells. It is readily detectable in the sera of patients with inflammatory rheumatic disease.[66,67] Abundant production of inducible HSP70 has been detected in synovial tissues and fluids of patients with RA.[67] The role of HSP70 in RA is rather controversial because it may confer both pro- and anti-inflammatory effects.[68,69]

HSP96 (gp96; tumor rejection antigen 1) is exclusively synthesized in the endoplasmic reticulum of vertebrate cells. Under normal conditions, the level of extracellular expression of HSP96 is low, but expression may be increased by hypoxia, stress, or malignancies.[70] This protein is a Toll-like receptor chaperone, and the cellular expression of HSP96 may induce a lupus-like syndrome and other autoimmune conditions through the sustained stimulation of macrophages and dendritic cells.[70,71] In a recent study, Huang et al[72] detected a higher level of expression of synovial HSP96 in the presence of RA than in the presence of OA or in controls. This protein is a potent activator of macrophages, and this activation is mediated through Toll-like receptor 2 signaling. HSP96 also significantly induces the transcription of tumor necrosis factor α and interleukin 8.

Ferritin, like haptoglobin, is a glycosylated serum protein that serves as an acute-phase reactant. Several investigators have reported that ferritin is abundantly produced in various autoimmune diseases, including RA.[73–75] Both light and heavy ferritin subunits have been detected in rheumatoid synovial tissues. Cells in the lining layer, as well as interstitial macrophages and fibroblasts, express ferritin.[76] Ferritin concentrations are higher in synovial fluids in the presence of RA than in synovial fluids in the presence of OA or sera in the presence of RA.[75] There is little information regarding the possible roles of CGI-150 protein, the guanine nucleotide–binding protein $\beta 4$ subunit, and hypothetical protein LOC51237 in inflammatory states such as arthritis.

In conclusion, we assessed the effects of LLLT on rheumatoid synovial tissues. Two important proteins, vimentin and α-enolase, have been implicated in citrullinated autoantigen-driven autoimmunity in RA. We showed for the first time that laser treatment suppressed the synovial expression of these 2 proteins. In addition, for 10 other proteins, including haptoglobin, ferritin, heat shock proteins, complement factors, and matrix components (eg, osteoglycin, lumican, fibromodulin), synovial expression after laser irradiation was different. The exact significance of these findings warrants clarification.

A limitation of the present study was the small number of participants. However, small sample sizes are not unusual in human proteomic studies because of the high costs and limited availability of human tissue samples from surgery. Our *ex vivo* results certainly cannot be directly translated to clinical practice. Further *ex vivo* and *in vivo* studies are needed.

On the basis of our results, it can be concluded that *ex vivo* LLLT may favorably alter protein expression in rheumatoid synovial tissues, decreasing the expression of autoantigens such as vimentin and α-enolase, both of which play roles in the pathogenesis of RA. A photochemical mechanism may be responsible for the favorable effects of percutaneous LLLT in RA. Low-level laser therapy may lead to reduced synthesis rather than increased lysis of these proteins. Intra-articular LLLT also may be a modality for treating certain rheumatoid joints via arthroscopy, but more proteomic studies of rheumatoid synovial membranes treated by percutaneous LLLT are needed.

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