Autoimmunity Against YKL-39, a Human Cartilage Derived Protein, in Patients with Osteoarthritis

JUN-ICHIRO TSURUHA, KAYO MASUKO-HONGO, TOMOHIRO KATO, MASAHIRO SAKATA, HIROSHI NAKAMURA, TAICHI SEKINE, MASAHARU TAKIGAWA, and KUSUKI NISHIOKA

ABSTRACT. ***Objective.*** Our previous study revealed that some patients with rheumatoid arthritis (RA) possessed autoantibodies to YKL-39, a cartilage related protein. We investigated whether patients with osteoarthritis (OA) also displayed autoimmunity to YKL-39.

Methods. Autoantibodies to recombinant YKL-39 as well as human cartilage glycoprotein-39 were detected by ELISA and Western blotting. The tested serum samples were derived from 117 patients with OA, 94 patients with RA, and 2 groups of 50 arthropathy-free healthy donors who matched the OA and RA groups for age and sex. We determined autoepitopes on YKL-39 using 3 overlapping fragments of YKL-39 (designated F1, F2, F3). T cell proliferation response to YKL-39 was analyzed using the ^{3}H-thymidine incorporation assay.

Results. Autoantibodies to YKL-39 were detected in 13 (11.1%) patients with OA and 11 (11.8%) with RA. In the epitope mapping, all the 3 fragments of YKL-39 were found to carry autoepitopes, but F1 was recognized most frequently. Proliferative responses of peripheral blood mononuclear cells against YKL-39 were detected in 6 (46%) of the 13 OA patients who were positive for the anti-YKL-39 autoantibodies and in 2 (17%) of the 11 antibody positive RA patients.

Conclusion. These results show that autoimmunity to YKL-39 in patients with OA was present at equal or somewhat higher frequency than in patients with RA. The cellular and humoral immune responses to YKL-39 may be involved in the pathological process of OA as well as RA. (J Rheumatol 2002;29:1459–66)

Key Indexing Terms:
YKL-39
AUTOANTIBODY
OSTEOARTHRITIS
T CELL IMMUNITY

From the Rheumatology, Immunology and Genetics Program, Institute of Medical Science, St. Marianna University School of Medicine, Kawasaki; Torii Pharmaceutical Company Ltd., Mitsubishi Kagaku Bio-Clinical Laboratories Inc., Tokyo; and Department of Biochemistry and Molecular Dentistry, Okayama University Dental School, Okayama, Japan.

Supported in part by grants-in-aid from the Ministry of Health and Welfare, the Ministry of Education, Science, and Culture of Japan; the Japan Rheumatism Foundation; Kanae Foundation for Life & Socio-Medical Science; Uehara Memorial Foundation; and the Kanagawa Nanbyo Foundation.

J-I. Tsuruha, MS, Rheumatology, Immunology and Genetics Program, Institute of Medical Science, St. Marianna University School of Medicine, Torii Pharmaceutical Company Ltd.; K. Masuko-Hongo, MD, PhD; T. Kato, MD, PhD, Rheumatology, Immunology and Genetics Program, Institute of Medical Science, St. Marianna University School of Medicine; M. Sakata, MS, Rheumatology, Immunology and Genetics Program, Institute of Medical Science, St. Marianna University School of Medicine, Torii Pharmaceutical Company Ltd.; H. Nakamura, MD, PhD, Rheumatology, Immunology and Genetics Program, Institute of Medical Science, St. Marianna University School of Medicine; T. Sekine, MS, Rheumatology, Immunology and Genetics Program, Institute of Medical Science, St. Marianna University School of Medicine, Mitsubishi Kagaku Bio-Clinical Laboratories Inc.; M. Takigawa, DDS, PhD, Department of Biochemistry and Molecular Dentistry, Okayama University Dental School; K. Nishioka, MD, PhD, Rheumatology, Immunology and Genetics Program, Institute of Medical Science, St. Marianna University School of Medicine.

Address reprint requests to Dr. T. Kato, Rheumatology, Immunology and Genetics Program, Institute of Medical Science, St. Marianna University, School of Medicine, 2-16-1 Miyamae-Ku, Kawasaki 216-8512, Japan. E-mail: t3kato@marianna-u.ac.jp

Submitted June 27, 2001; revision accepted January 15, 2002.

Osteoarthritis (OA) is a chronic joint disease widely distributed among aged populations. OA is characterized by cartilage surface irregularities such as fissures and fibrillation, loss of the cartilage matrix, patchy chronic synovitis, and osteophytes. Generally, OA has been accepted as a degenerative disease caused mainly by the aging process and by overloading of the joints. However, there is a growing body of evidence that supports an immunological involvement in the pathogenesis of OA. First, an infiltration of immunocompetent cells such as T lymphocytes, B lymphocytes, and monocytes/macrophages into the OA synovial tissue has been reported[1-4]. Second, immunoglobulins and immune complexes have been detected in OA cartilage occasionally[5]. Third, autoantibody production[6,7], an association with expression of the human leukocyte antigen (HLA)-A and -B haplotypes, and an association with other autoimmune diseases were documented in a subset of OA (i.e., nodular generalized OA[5,8,9]). Finally, 2 studies, including ours, have shown oligoclonal T cell expansion in synovial membranes in OA[10,11], suggesting activation and proliferation of a limited number of T cell clones with distinct antigen specificity. Taken together, these data indicate an involvement of T and B cell mediated immunological mechanisms in the pathogenesis of OA.

Indeed, the existence of autoimmune responses in OA was described earlier. For example, autoimmune responses

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2002. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

against collagens or proteoglycan in OA were reported[12-21]. In this regard, it was observed that small fragments of proteoglycan were released into the joint fluid at a higher level in early stage OA than in advanced OA[22,23]. This suggests that the cartilage components produced in OA during early degradation stages might be recognized as autoantigens, and that the subsequent immune responses to the wide range of joint derived autoantigens enhance the deteriorating process of joint destruction. Thus investigation of the antigenicity and arthritogenicity of the individual proteins that make up the joint structure would extend our understanding of the autoimmune aspects of OA.

Recently, 2 chondrocyte derived proteins have been identified. Human cartilage glycoprotein-39 (HC gp-39, YKL-40 or chondrex), a member of the chitinase protein family, was reported to be an autoantigen in rheumatoid arthritis (RA)[23]. Further, the concentrations of HC gp-39 in sera and synovial fluid are related to the degree of joint destruction observed in RA and OA[24,25]. YKL-39, which is produced by articular chondrocytes, is another member of the chitinase protein family and shares amino acid homology with HC gp-39[26]. However, unlike HC gp-39, the pathological characteristics of YKL-39 are poorly understood at present. Since the major source of HC gp-39 is thought to be the liver, and not chondrocytes[26], whereas expression of YKL-39 is mainly in cartilage chondrocytes, YKL-39 may be a more specific cartilage autoantigen than HC gp-39. In support of this suggestion, we recently found that a subset of patients with RA more frequently have autoantibodies to YKL-39 than to HC gp-39[27].

To extend our understanding of the immunological aspects of OA, we investigated the humoral and cellular autoimmunity to YKL-39 in patients with OA and compared the findings to those of patients with RA. Our results revealed existence of an immune response to YKL-39 in a subset of OA patients with a frequency similar to the immune response to YKL-39 in RA patients. These data thus suggest that an immune response to the cartilage derived protein contributes to joint degradation in both OA and RA.

MATERIALS AND METHODS

Patients and controls. One hundred seventeen patients with OA (101 women, 16 men, ages 54 to 96 yrs, mean age 78) and 94 patients with RA (78 women, 16 men, ages 39 to 86 yrs, mean age 67) were enrolled. Patients were studied at St. Marianna University, Kawasaki; Nishioka Clinic, Mie; Nihon Medical School, Tokyo; and the University of Mie, Mie, Japan. Patients with RA were different from those tested in our previous study[28]. As controls for the OA patients, 50 healthy individuals (42 women, 8 men, ages 70 to 89 yrs, mean age 78) with no obvious clinical features of arthritic diseases were studied. Another group of 50 healthy individuals (43 women, 7 men, ages 39 to 86 yrs, mean age 67) was used as a control for the RA patients. OA was diagnosed by criteria reported previously[28]. Severity of OA was evaluated by Kellgren-Lawrence radiographic grading[29]. RA was diagnosed according to the revised criteria of the American College of Rheumatology[31]. All the samples were obtained with informed consent, and the ethical committee of St. Marianna University School of Medicine approved this study.

Preparation of recombinant proteins for HC gp-39 and YKL-39. The recombinant proteins of HC gp-39 and YKL-39 used in this study (rHC gp-39 and rYKL-39, respectively) were prepared as described[27]. Briefly, 2 cDNA fragments encoding HC gp-39 and YKL-39 were amplified from RNA extracted from a human chondrosarcoma cell line (HCS-2/8)[31] by the reverse transcription polymerase chain reaction (PCR). For epitope analysis, YKL-39 was further subdivided into 3 fragment regions (F1, F2, and F3). The nucleotide sequences of the primers for PCR were as follows: F1, sense: 5′-TTTGGATCCTACAAACTGGTTTGCTACTTTACC-3′, antisense: 5′-AAAGTCGACAGTGAAATGAGTGTTTTCTTTCTG-3′; F2, sense: 5′-TTTGGATCCGTAAGCTGGATCTACCCAGATCAG-3′, antisense: 5′-AAAGTCGACGTGCCCATATGTGGGGATGCCCAT-3′; and F3, sense: 5′-TTTGGATCCATGCCATCAGAGAAGGTGGTCATG-3′, antisense: 5′-AAAGTCGACCAAGGAGCCAAGGCTTCTCTTGAC-3′ where the recognition sites for the restriction enzymes used are underlined. Nucleotide sequences of PCR products were confirmed by sequencing.

The cDNA fragments that encode F1 (390 bp), F2 (390 bp), and F3 (402 bp) regions were each subcloned into a plasmid expression vector of pMAL-eHis, which carried 6 consecutive histidine residues $(His)_6$ at the C-terminus of the cloning sites for affinity purification[27]. The 3 cDNA fragments were then subcloned into the plasmid expression vector pMAL-eHis. From these constructs, recombinant proteins containing an N-terminal fusion to a maltose-binding protein (MBP) and a C-terminal histidine tag were produced in *Escherichia coli*.

Enzyme linked immunosorbent assay. The ELISA to detect the autoantibodies was as described[32]. Antibody binding reactivity with a fusion protein, measured by ELISA, was expressed in binding units according to the formula: sample (binding units) = [OD sample/(mean OD sample + 3 SD of normal serum)] × 100. In each sample, the OD value of MBP was subtracted from the OD value of the fusion protein to obtain the OD of the sample. According to this formula, 100 binding units were used as the cutoff point [OD: optical density].

Western blotting. Western blotting was performed as described[33]. Briefly, 5 μg of the purified fusion proteins (HC gp-39, YKL-39, or F1, F2, F3 of YKL-39) or MBP were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto nitrocellulose membranes. After blocking and washing, the membranes were incubated with the respective serum samples. The membranes were then washed and incubated with horseradish peroxidase conjugated goat anti-human IgG (Zymed, San Francisco, CA, USA). Finally, the membrane-bound serum antibodies were visualized using diaminobenzidine as a substrate for the horseradish peroxidase.

T cell proliferation assay. Peripheral blood mononuclear cells (PBMC) were isolated by the standard gradient centrifugation method using Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden), and then maintained in RPMI medium supplemented with 10% fetal calf serum. The proliferation of PBMC after incubation with rHC gp-39, rYKL-39, or MBP was quantified using the [^{3}H] labeled thymidine assay[35]. For each sample, the counts per minute (cpm) value obtained by MBP stimulation was used as a background and subtracted from the stimulation obtained with the fusion protein. According to this formula, a 2-fold stimulation index (SI) was used as the positive cutoff point. Results are expressed as mean cpm or SI units.

RESULTS

Detection of autoantibodies to rHCgp-39 and rYKL-39 in OA and RA. We reported[27] the presence of autoantibodies to rHC gp-39 and rYKL-39 in 1% and 10%, respectively, of patients with RA. Here we extended our study to investigate the presence of the autoantibodies in patients with OA using the same series of recombinant proteins (Figure 1). To

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2002. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

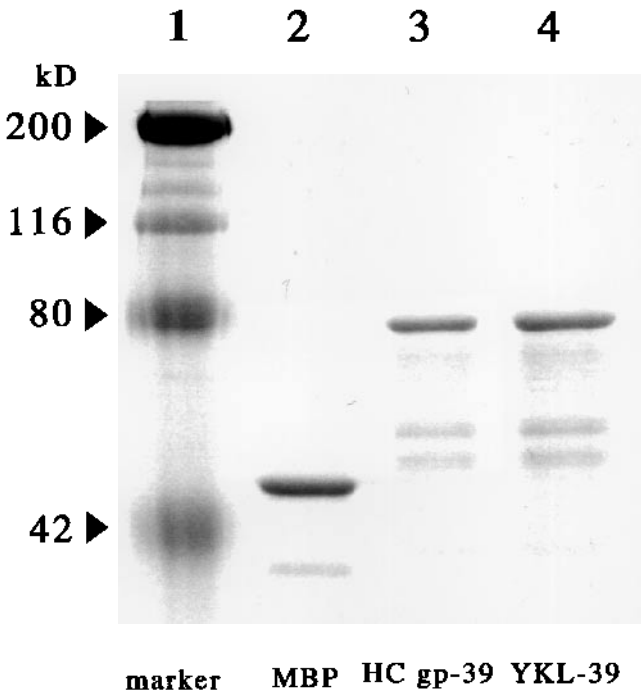


Figure 1. Purification of HC gp-39 and YKL-39 recombinant proteins. The recombinant proteins and maltose binding protein (MBP) were separated by 10% SDS-PAGE and stained with Coomassie blue. Lane 1: molecular weight markers. Lane 2: MBP as control. Lane 3: recombinant HC gp-39. Lane 4: recombinant YKL-39.

further generalize our earlier results from RA, RA patients of a different cohort from that tested in our previous study[27] were examined.

In agreement with our previous findings[27], we detected only a low frequency of autoantibodies against rHC gp-39: only one of the 117 OA serum samples (0.9%) and one of the 94 RA samples (1.1%) were positive (Figure 2A). In contrast, autoantibodies to rYKL-39 were detected in 13 (11.1%) of the 117 OA serum samples and 11 (11.8%) of the 94 RA serum samples (Figure 2B). None of the control sera were positive for anti-rYKL-39 or anti-rHC gp-39 (Figures 2A, 2B). The 2 samples positive for anti-rHC gp-39 (one in OA and one in RA; Figure 2A) were found to be negative for anti-rYKL-39 reactivity (Figure 2B), suggesting that the antigenicities of these 2 proteins were quite different. Thus, it was found that the frequency of autoantibodies to rYKL-39 was much higher than the frequency of autoantibodies to rHC gp-39 among both OA and RA patients.

To confirm the specific antibody reactivity to YKL-39 in the OA and RA patients, the autoantibodies in serially diluted serum samples were titrated by ELISA. As shown by the representative results in Figure 2C, the antibody reactivity to rYKL-39 increased in a serum concentration dependent manner, but the reactivity against MBP did not. Further, the existence of YKL-39-specific autoantibodies was also confirmed by Western blotting. As shown in Figure 2D, rYKL-39, but not MBP alone, was bound by the serum samples. This result indicates that there is a YKL-39-specific immune response in the OA and RA patients.

Epitope mapping of YKL-39. We next tried to determine the antigenic regions of rhYKL-39. For this purpose, we constructed 3 pMAL-eHis plasmids with cDNA fragments of F1, F2, or F3, which together covered the entire protein coding region of YKL-39 (Figure 3). These proteins were produced as MBP fusion proteins and then purified as shown in Figure 4A.

Serum samples of 17 patients who were positive for the anti-YKL-39 antibodies were then tested for reactivity to the 3 fragments using ELISA. The summary of the results is shown in Table 1. It was found that 2 of the 17 serum samples (11.8%) reacted to all fragments (OA-5, RA-1), while 12 (70.6%) serum samples reacted to only one fragment. In total, F1, F2, and F3 fusion proteins were recognized by 12 (70.6%), 7.5 (44.1%), and 3.5 (20.6%), respectively, of the 17 serum samples. When the reactivity was compared between anti-YKL positive OA and RA patients, the F1 fragment was found to carry autoepitopes of anti-YKL-39 in 53.8% of OA patients and in 50% of RA patients. The presence of each fragment autoantibody was also confirmed by Western blotting. As shown in Figure 4B, rYKL-39, but not MBP alone, was bound by the serum samples. This result indicates that there is a YKL-39-specific immune response in OA and RA patients, especially to epitope(s) in the F1 fragment.

Table 1. Summary of the reactivity of anti-YKL-39 antibody positive sera to the 3 truncated fusion proteins of YKL-39 by ELISA. Sample numbers denote individual patients.

Patient Sample	F1	F2	F3
OA-1	+	–	–
OA-2	–	+	–
OA-3	–	–	+
OA-4	+	±	–
OA-5	+	+	+
OA-6	+	–	–
OA-7	+	–	±
OA-8	+	–	–
OA-9	+	–	–
OA-10	+	–	–
OA-11	–	+	–
OA-12	+	+	–
OA-13	–	+	–
	(9/13)	(5.5/13)	(2.5/13)
RA-1	+	+	+
RA-2	–	+	–
RA-3	+	–	–
RA-4	+	–	–
	(3/4)	(2/4)	(1/4)

±: > mean OD + 2 SD of control, + : > mean OD + 3 SD of control.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2002. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

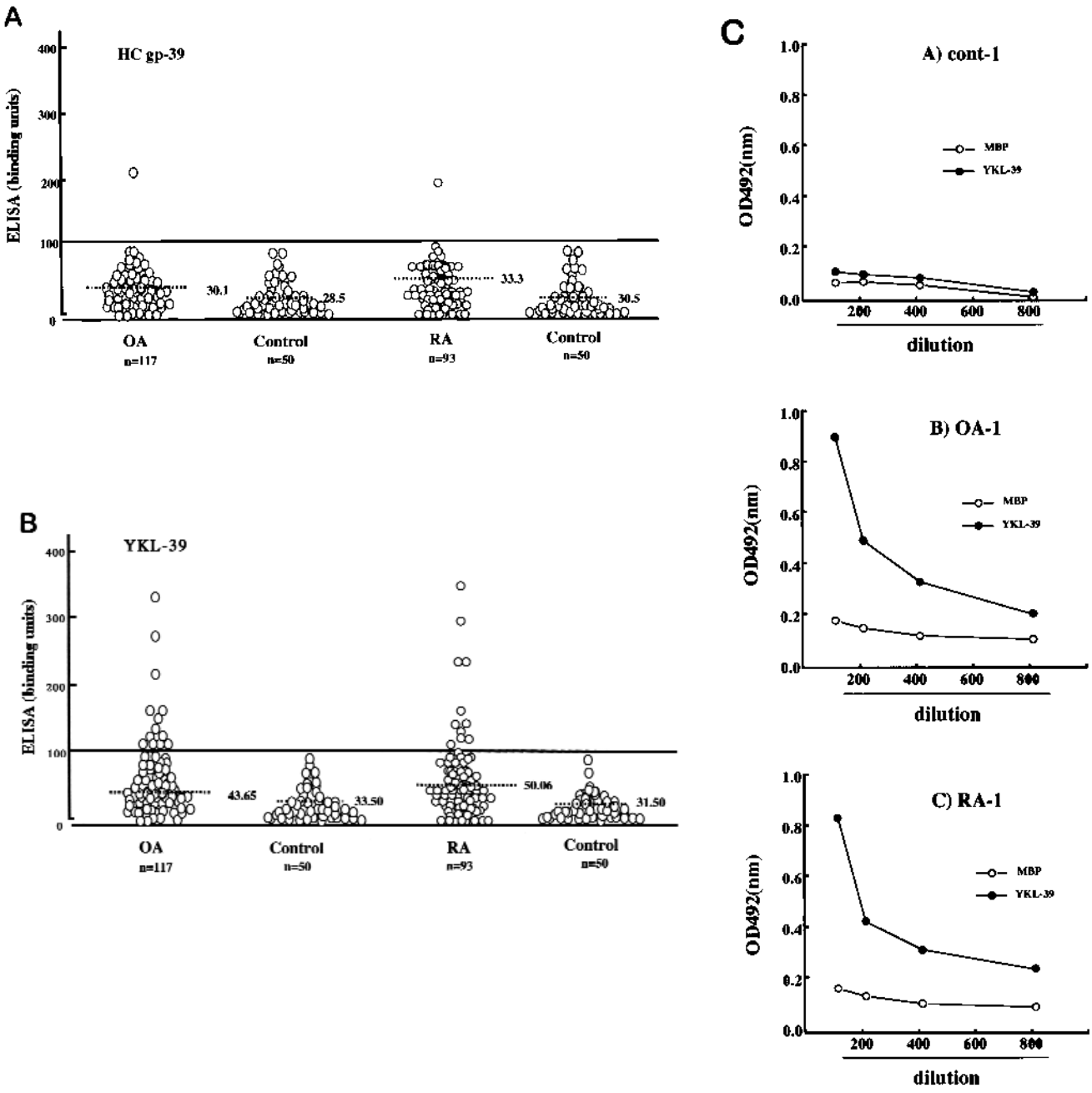


Figure 2. Autoantibody response to the chondrocyte produced proteins. A. Prevalence of antibodies to recombinant HC gp-39 by ELISA. B. Prevalence of antibodies to recombinant YKL-39 by ELISA. Each circle indicates a result from one serum sample. Results are expressed in binding units. Bars (100 binding units) represent the positive cutoff level. C. Specificity of the ELISA analysis. Six representative OA or RA serum samples that were positive for anti-YKL-39 antibody were serially diluted and analyzed by ELISA. Results from 2 representative cases, OA-1 and RA-1, are shown. One control sample was also analyzed. D (opposite page). Western blotting. Recombinant YKL-39 and the fusion partner MBP were separated by 10% SDS-PAGE. Serum samples with an ELISA positive anti-YKL-39 antibody titer were tested by immunoblotting. Control membranes were stained with Ponceau S. Four patients samples and control are shown.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2002. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

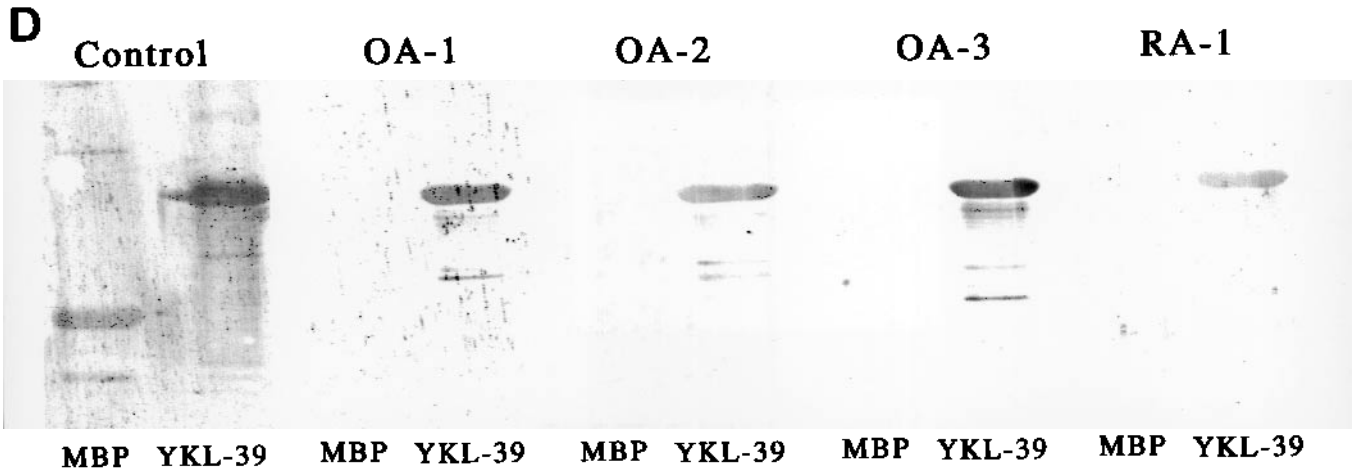


Figure 2d.

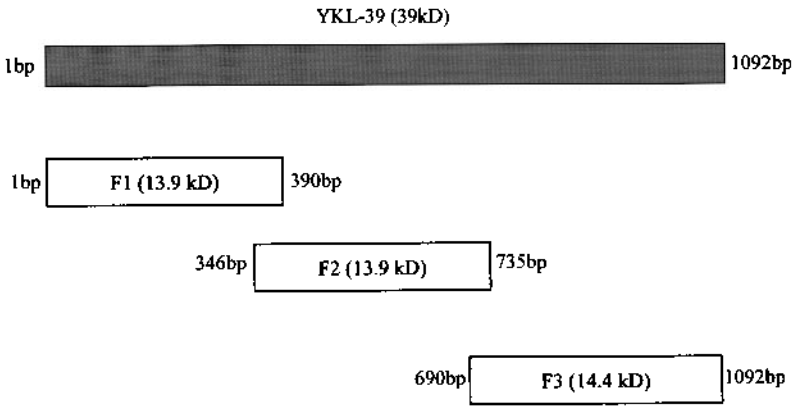


Figure 3. Constructs of YKL-39 fusion proteins (YKL-39, F1, F2, and F3). The numbers denote the nucleotide numbering of the human YKL-39 cDNA fragments that encoded YKL-39 (1092 base pairs, molecular weight 39 kDa), F1 (390 bp, MW 13.9 kDa), F2 (390 bp, MW 13.9 kDa), and F3 (402 bp, MW 14.4 kDa). All peptides were expressed as maltose binding protein (MBP) fusion proteins in *E. coli*.

T cell response to HC gp-39 and YKL-39. As autoantibodies to YKL-39 were detected in a subset of the OA and RA patients, we questioned whether a cellular response to YKL-39 was detectable in these patients. To this end, PBMC from the patients who were positive for the anti-YKL-39 antibody and from controls were stimulated with rYKL-39, rHC gp-39, or MBP, and then their proliferation responses were analyzed. PBMC from 6 (46.1%) of the 13 OA patients carrying the anti-YKL-39 antibody showed a significantly increased incorporation of [^{3}H] labeled thymidine in response to rYKL-39. In contrast, only 2 (16.7%) of the 11 RA patients carrying the antibody showed a T cell response to rYKL-39 (Figure 5 and Table 2). Thus, a detectable proliferative response of PBMC to YKL-39 was more frequent in the antibody positive patients with OA than in those with RA.

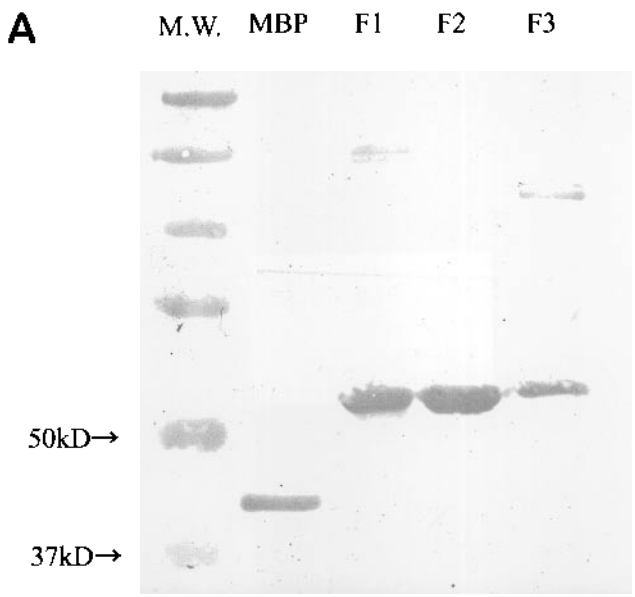
Clinical features of OA patients who carry anti-YKL-39 antibody. We compared the clinical features, including radiographic grading[29], of the anti-YLK-39 antibody positive and negative OA patients. As shown in Table 3, there was no significant correlation between the clinical grading and the presence of the anti-YKL-39 antibody. The laboratory data of the anti-YKL-39 antibody positive and negative patients also were not significantly different.

DISCUSSION

Our main findings are as follows: first, autoantibodies to YKL-39 were present in over 10% of the patients with OA and RA; second, autoantibodies to HC gp-39 were rare among the OA and RA patients; third, a proliferative response of PBMC to YKL-39 was detected more frequently in OA than in RA patients with anti-YKL-39 antibody; and finally, each fragment of YKL-39 carries autoepitopes, among which F1 was the most frequently recognized.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2002. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org



To our knowledge, this is the first report demonstrating that YKL-39 was recognized with similar frequencies in both OA and RA patients. Recent studies on cartilage related proteins have focused on their antigenic properties, and the proteins have been suggested to be involved in the immunity mediated pathophysiology of OA. Although there is no direct evidence that OA is caused by an autoimmune reaction, the pathological process of cartilage degradation may be enhanced by the immune responses occurring within the joint space. Indeed, a variety of cartilage or chondrocyte derived proteins are reported to be recognized as antigens in

Figure 4. Separation and analysis of fragment proteins. The purified F1, F2, and F3 fragment proteins and MBP were separated by 8% SDS-PAGE. A. The control membrane was stained with Ponceau S. B. Serum samples with an ELISA positive antibody titer against F1, F2, F3, or all 3 fragments were tested by immunoblotting. Autoantibody against YKL-39 fragments F1, F2, and F3 was detected in anti-YKL39 autoantibody positive OA and RA serum. Four representative samples are shown.

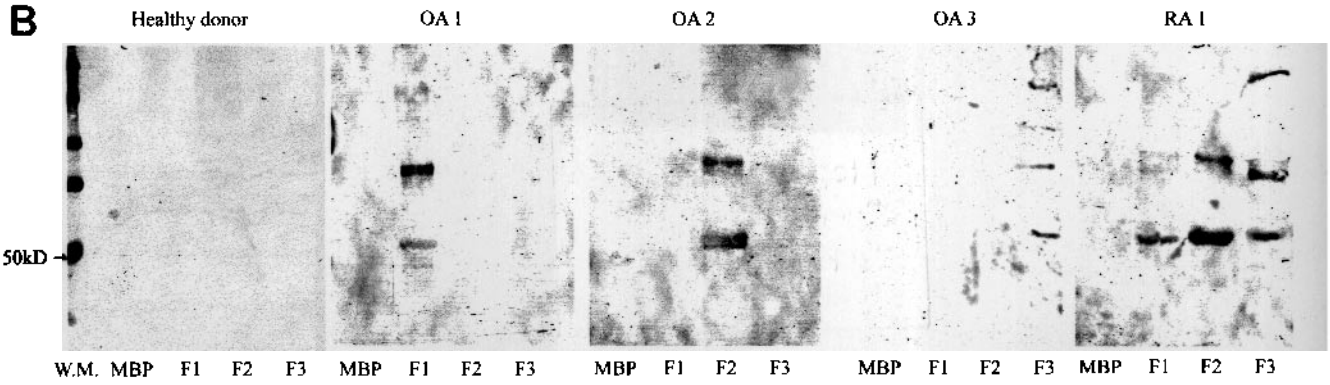


Table 2. Proliferative responses to YKL-39 in patients with osteoarthritis (OA), rheumatoid arthritis (RA), and healthy controls. Values are the median (range) stimulation index (SI) and counts per minutes (cpm).

	YKL-39	
	SI	CPM
Patients with OA, n = 13	2.06** (1.02–4.39)	2545 (52–5421)
Patients with RA, n = 11	1.49 (1.03–2.33)	3173 (35–4950)
Controls, n = 8	1.19 (0.73–1.97)	112 (51–282)

**: $p < 0.01$ Student's t test vs controls.

Table 3. Correlation of joint destruction by Kellgren-Lawrence radiographic grade [30] and frequency of anti-YKL-39 antibody in patients with OA.

Radiographic Grade	Positive, n = 9 (%)	Negative, n = 44 (%)
O	0 (0)	4 (9)
I	2 (22)	14 (32)
II	4 (44)	12 (27)
III	2 (22)	9 (20)
IV	1 (11)	5 (11)
Mean	2.25	1.93

OA. For example, in addition to proteoglycans and collagens, proteins such as cartilage oligometric matrix protein (COMP) or cartilage intermediate layer protein (CILP) are reported to be recognized in OA[35-37]. In regard to CILP, we reported that immunity to CILP in immunized mice may lead to cartilage pathology[37], suggesting an important contribution of such an immune response to the pathogenesis of arthropathy. Because the frequency of YKL-39 antigen recognition was similar in OA and RA (Figure 2B), but much lower in other autoimmune diseases[27], YKL-39 should be considered another candidate autoantigen with significant relevance to arthropathy.

The frequency of the autoantibody to rHC gp-39 was found to be around 1% in the patients with OA or RA. This frequency is as low as that seen in our previous study of a different group of RA patients[27]. These results appear to contrast with those of Verheijden, *et al*[23], who showed significant proliferation of peripheral blood T cells in response to a synthetic HC gp-39 derived peptide with a DR4 binding motif in 8 of 18 patients with RA (44%). There are several possible explanations for this apparent discrep-

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2002. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

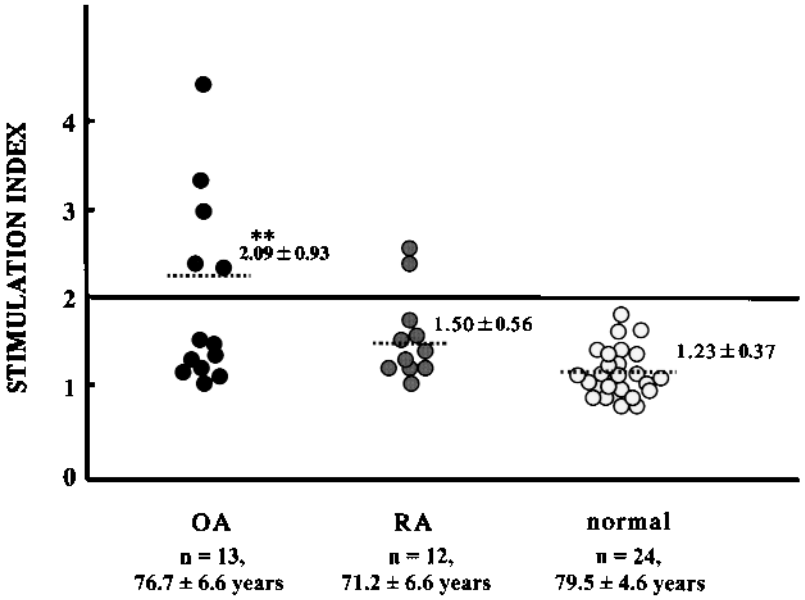


Figure 5. T cell proliferation in response to YKL-39 and HC gp-39 was tested in patients positive for the YKL-39 antibody and in controls. The bar indicates the cutoff value. The mean ± SD of the mean stimulation index in each group is shown. **p < 0.01, Student t test vs controls.

ancy. First, an autoimmune response to HC gp-39 may be directed toward cellular rather than humoral immunity. Second, the recombinant form of HC gp-39 used in our study may lack the natural epitope structure because of incorrect folding due to synthesis as a nonglycosylated form by *E. coli*. However, this explanation appears less likely, since rabbit anti-HC gp-39 antibodies generated against the native form of HC gp-39 (data not shown) reacted to rHC gp-39, indicating that it exhibits at least part of the natural epitope. Third, the peptide region tested by Verheijden, *et al* may be cryptic within the HC gp-39 molecule but be recognized by a cross-reaction of T cells, since T cell subpopulations apparently specific for one antigenic peptide have been shown to recognize other nonhomologous peptides. Thus, the responses observed by Verheijden, *et al* may not be wholly specific for HC gp-39.

Finally, we investigated the presence of epitopes in each fragment of YKL-39, utilizing 3 overlapping recombinant proteins. We found that the N-terminal F1 region was most frequently recognized in the serum of anti-YKL-39 antibody positive patients. Specifically, 69% of the OA and 75% of the RA patients who were positive for anti-YKL-39 antibodies reacted with the F1 fragment. Five patients also reacted with F2 and/or F3, in addition to F1. Only 5 of 17 patients reacted exclusively with F2 or F3. These data indicate that the F1 fragment does include a specific, immunodominant epitope of rhYKL-39, and that antigens are being recognized in the F2 and F3 fragments. To date, we have not found any amino acid sequence homology between F1 and other known antigens, nor any correlation between antigen recognition and patients' clinical status. The latter may be due to the small number of patients analyzed. Identification of the antigenic peptides within YKL-39, and especially within the F1 fragment, may lead to development of a therapy for arthropathy based on antigen-specific immunomodulation.

This is the first study to demonstrate the autoimmune responses to YKL-39 in both OA and RA patients. The specific immune response to a chondrocyte derived protein such as YKL-39 may be an important modulator of the pathologic process of cartilage degradation.

ACKNOWLEDGMENT

The authors are grateful to Dr. T. Matsui, St. Marianna University, and Dr. K. Sudo, Mie University, for providing clinical samples. We also appreciate the technical assistance of K. Furusawa and C. Furuya.

REFERENCES

1. Revell PA, Myston V, Lalor P. The synovial membrane in osteoarthritis: a histological study including the characterization of the cellular infiltrate present in inflammatory osteoarthritis. Ann Rheum Dis 1988;47:300-7.
2. Jasin HE. Autoantibody specificities of immune complexes sequestered in articular cartilage of patients with rheumatoid arthritis and osteoarthritis. Arthritis Rheum 1985;28:241-8.
3. Cooke TDV, Richer S, Hurd E, Jasin HE. Relationships of immune deposits in articular cartilaginous tissues to synovitis in osteoarthritis. J Rheumatol 1983;10 Suppl 9:55-6.
4. Sakkas LI, Scanzello C, Johanson N, et al. T cells and T-cell cytokine transcripts in the synovial membrane in patients with osteoarthritis. Clin Diagn Lab Immunol 1998;5:430-7.
5. Pattrick M, Manhiere A, Milford-Ward A, Doherty M. HLA-A, B antigens and alpha-1-antitrypsin phenotypes in nodal generalized osteoarthritis. Ann Rheum Dis 1989;48:470-5.
6. Hopkinson ND, Powell RJ, Doherty M. Autoantibodies, immunoglobulins and Gm allotypes in nodal generalized

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2002. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org

osteoarthritis. Br J Rheumatol 1992;31:605-8.
7. Mollenhauer J, von der Mark K, Burmester G, et al. Serum antibodies against chondrocyte cell surface proteins in osteoarthritis and rheumatoid arthritis. J Rheumatol 1988;15:1811-7.
8. Doherty M, Patrick M, Powell RJ. Hypothesis — nodal generalized osteoarthritis is an autoimmune disease. Ann Rheum Dis 1990;49:1017-20.
9. Shuckett R, Russel ML, Gladman DD. A typical erosive osteoarthritis and Sjogren syndrome. Ann Rheum Dis 1986;45:281-8.
10. Stamenkovic I, Stegagno M, Wright KA, et al. Clonal dominance among T-lymphocyte infiltrates in arthritis. Proc Natl Acad Sci USA 1988;85:1179-83.
11. Nakamura H, Yosino S, Tsuruha J, et al. T-cell mediated inflammatory pathway in osteoarthritis. Osteoarthritis Cartilage 1999;7:401-2.
12. Charriere G, Hartmann DJ, Vignon E, Ronziere M-C, Herbage D, Ville G. Antibodies to Types I, II, IX, and XI collagen in the serum of patients with rheumatic diseases. Arthritis Rheum 1988;31:325-32.
13. Poll AR. Immunology of cartilage. In: Moskowitz RW, Howell DS, Goldberg VM, Mankin HJ, editors. Osteoarthritis diagnosis and medical/surgical management. 2nd ed. Philadelphia: WB Saunders; 1992:155-89.
14. Golds EE, Cooke TD, Poole AR. Immune regulation of collagenase secretion in rheumatoid and osteoarthritic synovial cell cultures. Coll Relat Res 1983;3:125-40.
15. Neame PJ, Christner JE, Baker JR. Cartilage proteoglycan aggregates: the link protein and proteoglycan amino-terminal globular domains have similar structures. J Biol Chem 1987;262:17768-78.
16. Barry FP, Rosenberg LC, Gaw JU, Koob TJ, Neame PJ. N- and O-linked keratan sulfate on the hyaluronan binding region of aggrecan from mature and immature bovine cartilage. J Biol Chem 1995;270:20516-24.
17. Heinegard D, Axelsson I. Distribution of keratan sulfate in cartilage proteoglycans. J Biol Chem 1977;252:1971-9.
18. Fosang AJ, Hardingham TE. Isolation of the N-terminal globular protein domains from cartilage proteoglycans: identification of G2 domain and its lack of interaction with hyaluronate and link protein. Biochem J 1989;261:801-9.
19. Glant TT, Mikecz K, Thonar EJM, Kuettner KE. Immune responses to cartilage proteoglycans in inflammatory animal models and human disease. In: Woessner JF Jr, Howell DS, editors. Joint cartilage degradation: basic and clinical aspects. New York: Marcel Dekker; 1993:435-73.
20. Karopoulos C, Rowley MJ, Ilic MZ, Handley CJ. Presence of antibodies to native G1 domain of aggrecan core protein in synovial fluids from patients with various joint diseases. Arthritis Rheum 1996;39:1990-7.
21. Guerassimov A, Zhang Y, Banerjee S, et al. Autoimmunity to cartilage link protein in patients with rheumatoid arthritis and ankylosing spondylitis. J Rheumatol 1998;25:1480-4.
22. Bari AS, Carter SD, Bell SC, Morgan K, Bennet D. Anti-type II collagen antibody in naturally occurring canine joint diseases. Br J Rheumatol 1989;28:480-6.
23. Verheijden GFM, Rijnders AWM, Bos E, et al. Human cartilage glycoprotein-39 as a candidate antigen in rheumatoid arthritis. Arthritis Rheum 1997;40:1115-25.
24. Johansen JS, Stoltenberg M, Hansen M, et al. Serum YKL-40 concentrations in patients with rheumatoid arthritis: relation to disease activity. Rheumatology 1999;38:618-26.
25. Volck B, Ostergaard K, Johansen JS, Garbausch C, Price PA. The distribution of YKL-40 in osteoarthritic and normal human cartilage. Scand J Rheumatol 1999;28:171-9.
26. Hu B, Trich K, Fignenia WF, Prince PA. Isolation and sequence of a novel human chondrocyte protein related to mammalian members of chitinase protein family. J Biol Chem 1996;271:19415-20.
27. Sekine T, Masuko-Hongo K, Matsui T, et al. YKL-39, a human cartilage-related protein, is recognized as a target antigen in patients with rheumatoid arthritis. Ann Rheum Dis 2001;60:49-54.
28. Altman RD. The classification of osteoarthritis. J Rheumatol 1995;22 Suppl 43:42-3.
29. Kellgren JH, Lawrence JS. Radiological assessment of osteoarthritis. Ann Rheum Dis 1957;16:494-501.
30. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
31. Yoichi O, Yuso G, Yusuke K, et al. Purification of an angiogenesis inhibitor from culture medium conditioned by human chondrosarcoma-derived chondrocytic cell lines HCS-2/8. Biochem Biophys Acta 1995;1245:1-8.
32. Matsui T, Kurokawa M, Kobata T, et al. Autoantibodies to T cell costimulatory molecules in systemic autoimmune diseases. J Immunol 1999;162:4328-35.
33. Yamamoto K, Miura H, Moroi Y, et al. Isolation and characterization of a complementary DNA expressing human U1 small nuclear ribonucleoprotein C polypeptide. J Immunol 1988;140:311-7.
34. Guerassimov A, Zhang Y, Cartman A, et al. Immune responses to cartilage link protein and the G1 domain of proteoglycan aggrecan in patients with osteoarthritis. Arthritis Rheum 1999;42:527-33.
35. Lohmander LS, Saxne T, Heinegard DK. Release of cartilage oligomeric matrix protein (COMP) into joint fluid after knee injury and in osteoarthritis. Ann Rheum Dis 1994;53:8-13.
36. Lorenzo P, Bayliss M, Heinegard D. A novel cartilage protein (CILP) present in the mid-zone of human articular cartilage increases with age. J Biol Chem 1998;36:23463-8.
37. Tsuruha J, Masuko-Hongo K, Kato T, Sakata M, Nakamura H, Nishioka K. Implication of cartilage intermediate layer protein (CILP) in cartilage destruction in subsets of patients with osteoarthritis and rheumatoid arthritis. Arthritis Rheum 2001; 44:838-45.

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2002. All rights reserved.

Downloaded on April 20, 2024 from www.jrheum.org