Autoantibodies to Osteopontin in Patients with Osteoarthritis and Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Osteopontin (OPN), secreted mainly from chondrocytes, is suggested to be involved in the ossification and remodeling of bone and also in regulation of cytokine profiles. We investigated whether patients with osteoarthritis (OA) and rheumatoid arthritis (RA) display autoimmunity against OPN.

Methods. Recombinant human OPN (rhOPN) was prepared as a fusion protein with ß-galactosidase using *E. coli*. Serum samples from patients with OA or RA and from age matched healthy donors were tested for autoantibodies to rhOPN using ELISA and Western blotting. Reactivity of the same samples to purified native human OPN (nhOPN) was investigated by ELISA separately, to evaluate conformational epitopes.

Results. By ELISA, autoantibodies to rhOPN were found in one (0.95%) of 105 patients with OA and 2 (2.3%) of 88 patients with RA. These autoantibodies to rhOPN were confirmed by Western blotting. In contrast, 11 (9.5%) of 105 OA serum and 13 (15%) of 88 RA serum samples reacted to nhOPN. The anti-OPN positive RA patients showed high serum levels of rheumatoid factor and C-reactive protein and accelerated erythrocyte sedimentation rate compared to the anti-OPN negative group, although the differences did not achieve statistical significance.

Conclusion. Our data showed that OPN is one of the autoantigens in OA and RA. Preferential recognition of nhOPN to rhOPN indicates that major epitope(s) of OPN would be conformational. Clinically, existence of the anti-OPN antibodies may be linked to disease severity in RA. (J Rheumatol 2001;28:1492–5)

Key Indexing Terms:
OSTEOPONTIN AUTOANTIGEN RHEUMATOID ARTHRITIS OSTEOARTHRITIS

Several lines of study have revealed autoimmune responses against various cartilage related proteins in rheumatoid arthritis (RA) and osteoarthritis (OA)[1,2]. For instance, autoantibodies to type II collagen were reported to be detected in up to 30% of patients with RA[3], and more recently, a chondrocyte-secreted protein of YKL-40 was reported to be an autoantigen in RA[4]. Several cartilage related proteins including these 2 proteins were shown to cause polyarthritis when mice were immunized with them[3,4]. Thus autoimmunity to cartilage related proteins is thought to be deeply involved in the pathogenesis of RA. Further, some of these proteins were reported to be autoantigens in patients with osteoarthritis (OA)[5,6]. In this context, autoimmunity to other cartilage related proteins should be investigated in patients with RA and OA.

Osteopontin (OPN) is expressed by various cells and tissues like kidney, placenta, neoplastic cells, lining epithelial cells, islet cells, and activated T cells; however, the main source of OPN is bone cells, including chondrocytes and osteoclasts[7,8]. OPN is an acidic calcium-binding glycosylated phosphoprotein and is one of the major noncollagenous proteins in bone[6]. Since OPN is expressed mainly in the endochondral ossification site as well as the remodeling region of bone[6,9,10], it is suggested that OPN has a role in maintaining physiological bone structure. OPN interacts with integrin receptors through its Arg-Gly-Asp (RGD) motif and thus may assist cellular attachment to the extracellular matrix[6]. Immunologically, OPN binds to an adhesion molecule of CD44, which is known to be overexpressed in inflammatory joints[11]. Further, OPN transgenic mice displayed increased titers of anti-DNA antibodies[12]. Moreover, OPN deficient mice showed impaired type-1 immunity to viral and bacterial infection, indicating OPN was important in developing efficient type-1 immunity[13]. Based on these data, autoimmunity to OPN may have potential to modulate physiopathology of arthropathy including RA and OA. To clarify the autoimmunity of OPN, we inves-

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tigated whether autoantibodies to OPN exist in patients with RA and OA, using full length recombinant human OPN (rhOPN) and purified native human OPN (nhOPN).

MATERIALS AND METHODS

Serum samples. Serum samples were obtained from 105 patients with OA (ages 54–96 yrs, median 78; 104 women, 11 men), 88 patients with RA (ages 39–86, median 67; 78 women, 10 men), and 55 individuals with no manifestation of arthritic disease as a control group (ages 70–89, median 78; 43 women, 7 men). All participants provided informed consent for this study. The protocol was approved by our institutional ethical committee.

Recombinant and native human OPN. Chondrocytes were obtained from therapeutic surgery of a knee joint in a patient with trauma. Total RNA extracted from the cultured chondrocytes was reverse transcribed to cDNA using oligo-dT priming. The primers used were designed based on the reported nucleotide sequences of hOPN (894 bp)[14]. The amplified DNA was subcloned into a plasmid expression vector of pTEX-2-His, which had a continuous stretch of histidines (His) for affinity purification. In this expression system, rhOPN was expressed as a fusion protein with ß-galactosidase (ß-gal). The nucleotide sequence of the cloned DNA was confirmed to be that for hOPN by dideoxy sequencing. The rhOPN protein was produced in *Escherichia coli* and was purified as described[15]. The purified nhOPN was purchased from Sangi Inc., Tokyo, Japan.

ELISA and Western blotting. Microtiter plates were coated in 100 μl of 100 mM carbonate buffer, pH 9.4, that contained 2.0 μg/ml of nhOPN, rhOPN, or ß-gal at 4°C. The remaining ELISA procedure was as described[15]. In Western blotting, 5 μg of the purified rhOPN or ß-gal per lane was separated by 8% SDS-PAGE. The remaining procedure was similar to that described[15].

RESULTS

Expression of rhOPN. First, the purified rhOPN was separated by 8% SDS-PAGE and then was stained with Coomassie brilliant blue. This procedure revealed 2 major bands (Figure 1A). The upper band possessed an expected molecular weight of roughly 180 kDa and reacted with anti-ß-gal antibody and Ni-NTA, which bound to the histidine stretch at the end of the hOPN part (Figure 1B and data not shown). Thus this band was concluded to represent expression of the entire protein coding region of hOPN. The lower band, reacting with anti-ß-gal but not with Ni-NTA (Figure 1B and data not shown), was thought to be degraded products of the full length fusion protein or products of incomplete translation.

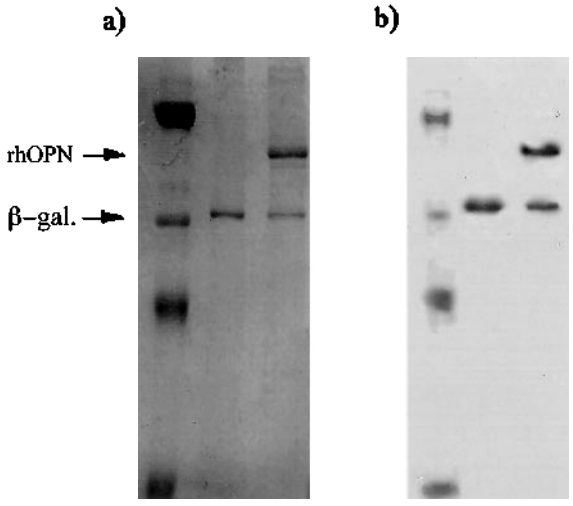

Figure 1. Expression and purification of rhOPN fusion protein. hOPN was produced as a fusion protein with ß-galactosidase (ß-gal) in *E. coli*. Purified hOPN fusion protein and ß-gal of a fusion partner were separated by 8% SDS-PAGE and stained with Coomassie brilliant blue (a). The separated fusion proteins were transferred onto nitrocellulose membranes, then were reacted with anti-ß-gal antibodies (b).

Detection of autoantibodies to rhOPN and nhOPN in patients with arthropathy. Using the rhOPN described above, we investigated whether autoantibodies to rhOPN were produced in patients with OA or RA. For this aim, 105 serum samples from patients with OA, 88 serum samples from patients with RA, and 50 serum samples from healthy donors were tested for their reactivity to rhOPN by ELISA. As a result, antibodies to rhOPN were detected in one out of the 105 OA serum samples (0.95%) and in 2 of 88 RA serum samples (2.3%) (Figure 2A). In ELISA that measured anti-rhOPN autoantibody titers in serially diluted serum samples of the 3 positive patients, the antibody titers to rhOPN increased in a serum concentration dependent manner, while antibody titers to ß-gal did not. Representative results are shown in Figure 2B. This indicates that the immune reaction was specific for rhOPN. In addition, the 3 positive serum samples also stained rhOPN but not ß-gal in Western blotting, as shown in Figure 2C. This also confirmed the anti-rhOPN reactivity.

To evaluate conformational epitopes on OPN, we performed ELISA using nhOPN. Importantly, this showed that 11 (9.5%) of the 105 OA serum and 13 (15%) of the 88 RA serum samples reacted to nhOPN (Figure 2D). The above 3 serum samples that reacted to rhOPN also reacted to nhOPN (closed circles in Figure 2D). This finding indicates that the major epitope(s) of OPN would not be linear but conformational.

Based on the ELISA results from nhOPN, we compared patients' clinical variables between anti-OPN positive and negative groups. In RA, we compared serum levels of rheumatoid factor (RF) and C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). All the 3 values were increased or accelerated in the anti-OPN positive group compared to the negative group (RF 136.7 ± 160.6 vs 90.7 ± 164.9 IU/ml; CRP 2.9 ± 2.9 vs 1.9 ± 2.8 mg/dl; ESR 35.2 ± 21.7 vs 31.7 ± 25.3 mm, all mean ± SD), although the differences did not achieve statistical significance. Radiographic staging of the joint destruction classified by the criteria of Steinbrocker, *et al* in RA and that of Kellgren-Lawrence in OA was not significantly different between the anti-OPN antibody positive and negative patients in both RA and OA (data not shown).

DISCUSSION

As described above, OPN is reported to be involved in endo-

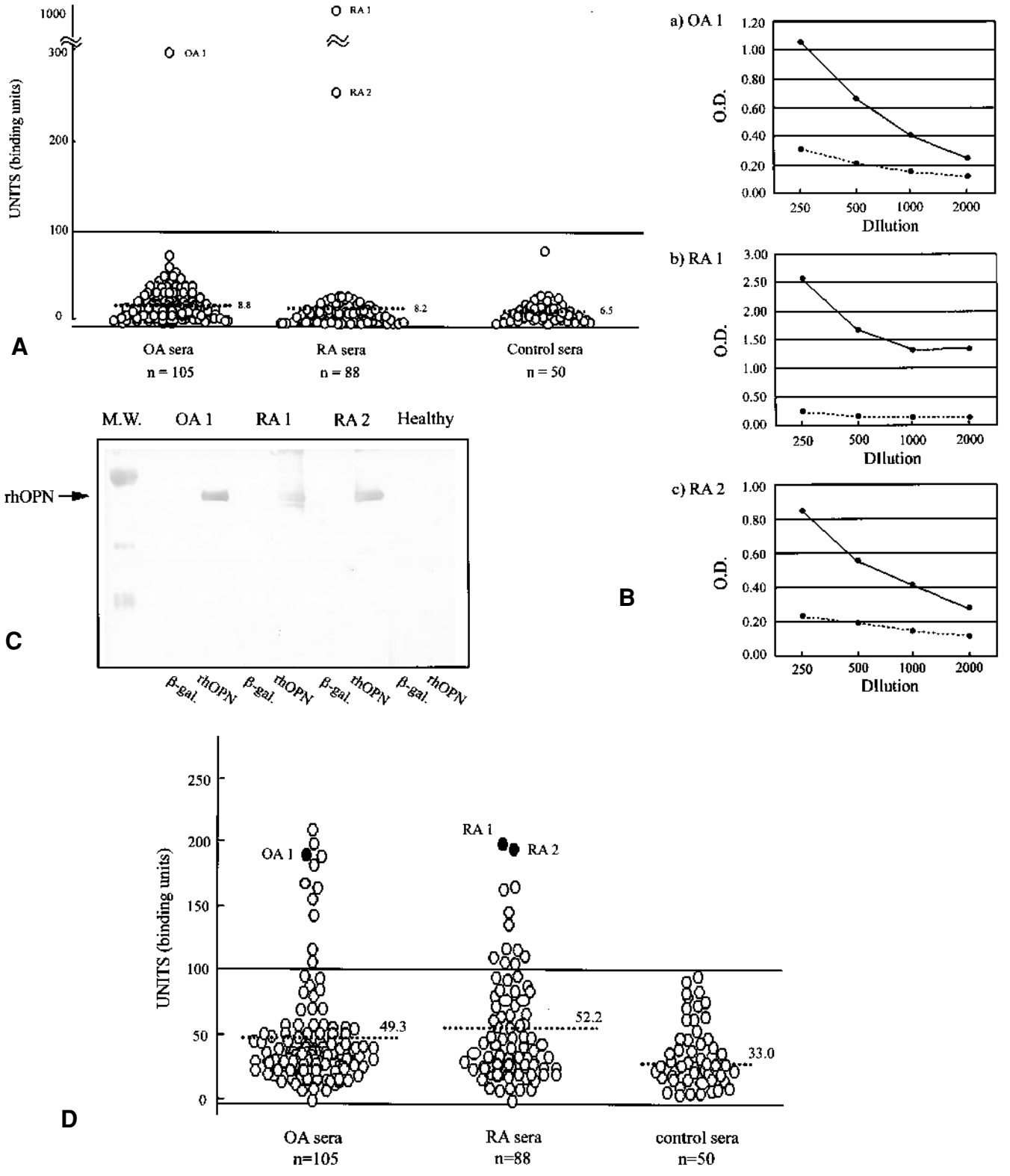

Figure 2. Autoantibody to OPN in arthritic patients. A. Prevalence of antibodies to rhOPN in sera from patients with OA, RA, and controls by ELISA. Serum dilution was 1/200. Optical density of each sample is indicated in binding units[15]. Bars represent the positive cutoff line. B. Results of ELISA for the anti-rhOPN antibody in sequentially diluted sera obtained from anti-rhOPN antibody positive patients. Solid lines indicate titers against rhOPN; broken lines: ß-gal. C. Reactivity of positive serum samples by ELISA was confirmed by Western blot. D. Prevalence of antibodies to nhOPN determined by ELISA, using the same serum samples and under the same conditions as in A. Closed circles indicate the serum samples that reacted to rhOPN.

chondral ossification and remodeling of the bone as well as in cellular attachment in the inflammatory area. Thus dysfunction of OPN may lead to degenerative joint diseases like OA, and may play some role in the pathogenesis of inflammatory joint diseases like RA. We investigated whether autoimmunity to OPN exists in patients with OA and RA. We observed production of autoantibodies to OPN in 9.5% of OA and 15% of RA patients tested. Recently, autoantibodies to OPN were reported in patients with insulin dependent diabetes mellitus (IDDM)[10]. The frequency was reported to be 7.8% in the patients with IDDM and 3.9% in healthy controls[10]. The prevalence of anti-OPN autoantibody was higher in patients with OA or RA, and thus anti-OPN autoantibody may be linked to the pathogenic process of the arthropathies.

It is of interest whether the anti-OPN autoantibodies modify clinical features of RA or OA by altering the functions of OPN. In this regard, the presence of the anti-OPN antibody appeared to be linked to severe disease conditions in RA, i.e., increased serum levels of RF and CRP, and accelerated ESR. However, the difference of each value was not statistically significant. Since OPN has multiple functions, autoantibodies to different epitopes on the OPN molecule may modulate the functions of OPN differently, and thus the effects of the autoantibodies may be different between individuals. Epitope analysis in a larger number of patients with anti-OPN autoantibodies will elucidate the clinical significance more precisely.

In addition, most of the anti-OPN positive serum samples reacted to the native OPN but not to recombinant human OPN. Thus a native form of OPN should be used to survey anti-OPN autoantibodies.

REFERENCES

1. Cope AP, Sfnderstrup G. Evaluating candidate autoantigens in rheumatoid arthritis. Springer Semin Immunopathol 1998;20:23-39.
2. Jasin HE. Immune mechanisms in osteoarthritis. Semin Arthritis Rheum 1989;18:86-90.
3. Banerjee S, Luthra HS, Moore SB, O'Fallon WM. Serum IgG anti-native type II collagen antibodies in rheumatoid arthritis: association with HLA DR4 and lack of clinical correlation. Clin Exp Rheumatol 1988;6:373-80.
4. Verheijden GFM, Rijnders AWM, Bos E, et al. Human cartilage glycoprotein-39 as a candidate autoantigen in rheumatoid arthritis. Arthritis Rheum 1997;40:1115-25.
5. Jasin HE. Autoantibody specificities of complexes sequestered in articular cartilage of patients with rheumatoid arthritis and osteoarthritis. Arthritis Rheum 1985;28:241-8.
6. Heinegard D, Oldberg A. Structure and biology of cartilage and bone matrix noncollagenous macromolecules. FASEB J 1989;3:2042-51.
7. Brown LF, Berse B, van de Water L, et al. Expression and distribution of osteopontin in human tissues: widespread association with luminal epithelial surfaces. Mol Biol Cell 1992;3:1169-80.
8. Fierabracci A, Biro PA, Yiangou Y, et al. Osteopontin is an autoantigen of the somatostatin cells in human islets: identification by screening random peptide libraries with sera of patients with insulin-dependent diabetes mellitus. Vaccine 2000;18:342-54.
9. Denhardt DT, Guo X. Osteopontin: a protein with diverse functions. FASEB J 1993;7:1475-82.
10. Dodds RA, Connor JR, James IE, et al. Human osteoclasts, not osteoblasts, deposit osteopontin onto resorption surfaces: an in vitro and ex vivo study of remodeling bone. J Bone Miner Res 1995;10:1666-80.
11. Naot D, Sionov RV, Ish-Shalom D. CD44: structure, function, and association with the malignant process [review]. Adv Cancer Res 1997;71:241-319.
12. Iizuka J, Katagiri T, Tada N, et al. Introduction of an osteopontin gene confers the increase in B1 cell population and the production of anti-DNA autoantibodies. Lab Invest 1998;78:1523-33.
13. Ashkar S, Weber GF, Panoutsakopoulou V, et al. Eta-1 (Osteopontin): An early component of type-1 (cell-mediated) immunity. Science 2000;287:860-4.
14. Hijiya N, Setoguchi M, Matsuura K, Higuchi Y, Akizuki S, Yamamoto S. Cloning and characterization of the human osteopontin gene and its promoter. Biochem J 1994;303:255-62.
15. Matsui T, Kurokawa M, Kobata T, et al. Autoantibodies to T cell costimulatory molecules in systemic autoimmune diseases. J Immunol 1999;162:4328-35.