Overexpression of Osteopontin in Rheumatoid Synovial Mononuclear Cells Is Associated with Joint Inflammation, Not with Genetic Polymorphism

GUANGWU XU, WEI SUN, DONGYI HE, LI WANG, WENXIN ZHENG, HONG NIE, LIQING NI, DONGQING ZHANG, NINGLI LI, and JINGWU ZHANG

ABSTRACT. ***Objective***. Osteopontin (OPN) is thought to play an important role in rheumatoid synovitis. We investigated the expression of OPN in rheumatoid synovial fluid mononuclear cells (SFMC) and its potential association with genetic polymorphism of the OPN gene and joint inflammation in rheumatoid arthritis (RA).

Methods. 1. The expression of OPN mRNA in peripheral blood mononuclear cells (PBMC) and SFMC of patients with RA was analyzed quantitatively by real-time polymerase chain reaction (PCR). Results were analyzed in paired PBMC and SFMC and control PBMC. 2. Six single nuclear acid polymorphisms of the OPN gene were genotyped in a cohort of 192 Chinese patients with RA and controls (n = 288) by restriction fragment length polymorphism PCR or direct DNA sequencing. 3. SF derived from RA patients was examined for the stimulating effect on mRNA expression of the OPN gene in PBMC.

Results. The expression of OPN gene was significantly increased in SFMC and, to a lesser degree, in PBMC of patients with RA compared to control PBMC ($p < 0.01$). However, the prevalence of OPN genotype and allele frequencies at the selected positions did not differ significantly between RA patients and the control group ($p > 0.05$). Further characterization indicated that SF known to contain a variety of proinflammatory factors significantly stimulated mRNA expression of OPN in PBMC obtained from RA patients or healthy controls.

Conclusion. Overexpression of OPN mRNA in SFMC is associated with proinflammatory factors produced in inflamed joints, but not with OPN genetic polymorphisms. OPN gene polymorphisms do not correlate with susceptibility to RA. (J Rheumatol 2005;32:410–6)

Key Indexing Terms:
OSTEOPONTIN
RHEUMATOID ARTHRITIS
SINGLE NUCLEOTIDE POLYMORPHISM
CYTOKINES

Osteopontin (OPN) is one of the major noncollagenous bone matrix proteins produced by osteoblasts and osteoclasts[1,2]. The human gene of OPN is located on chromosome 4q21–25[2]. The OPN gene is also expressed in T cells early in the course of bacterial infections (within 48 h), and interaction of its protein product with macrophages can induce inflammatory responses[3,4]. There is evidence suggesting that OPN acts as a proinflammatory cytokine and plays an important role in the regulation of tissue repair and inflammation[5,6]. Further, OPN stimulates T cell proliferation[7] and enhances the production of interferon-γ (IFN-γ) and interleukin 12 (IL-12), while it inhibits the production of IL-10, an antiinflammatory cytokine[8]. These lines of evidence indicate OPN is critical to recruitment of Th1 cells and upregulation of cytokine production in Th1-mediated cellular immunity.

Rheumatoid arthritis (RA) is a chronic inflammatory disease of the joint characterized by synovial inflammation and hyperplasia leading to progressive cartilage and bone destruction. Proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and IL-1, have been found to

From the Health Science Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai Second Medical University, Shanghai Institute of Immunology and E-Institute of Shanghai Universities, Shanghai; Guanghua Rheumatology Hospital, Shanghai, China; and Department of Immunology, Baylor College of Medicine, Houston, Texas, USA.

Supported by grants from Chinese Academy of Sciences Knowledge Innovation Program (KSCX2-SW-212) and the National High Technology Research and Development Program of China (863 Program 2002AA216121), National Natural Science Foundation (30430650), and Shanghai Commission of Science and Technology (04DZ14902, 04DZ19202, 04JC14040, 03 XD 14015).

G-W. Xu, MD, PhD; W. Sun, MD, PhD; W-X. Zheng, MD; H. Nie, MD, PhD; N-L. Li, MD, PhD, Health Science Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai Institute of Immunology; D-Y. He, MD; L-Q. Ni, MD, Guanghua Rheumatology Hospital; L. Wang, BS; D-Q. Zhang, MD, PhD, Shanghai Institute of Immunology, Shanghai Second Medical University; J-W. Zhang, MD, PhD, Health Science Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai Institute of Immunology, Shanghai Second Medical University E-Institute of Shanghai Universities, Department of Immunology, Baylor College of Medicine.

Address reprint requests to Dr. J-W. Zhang, Department of Neurology, Baylor College of Medicine, One Baylor Plaza, Mail Station NB302, Houston, TX 77030. E-mail: jzang@bcm.tmc.edu

Received July 13, 2004; revision accepted October 12, 2004.

play an important role in the disease process of RA[9]. Antagonism to TNF-α and IL-1 receptors has a therapeutic effect in RA. However, the pathological process of RA is complex and there are other factors critically involved in perpetuation of rheumatoid synovitis[10-12]. Several studies have suggested an important role for OPN in the pathogenesis of inflammatory arthritis. OPN-deficient rodents (OPN–/–) became resistant to type II collagen-induced arthritis[13]. Expression of OPN mRNA and its protein has been detected in synovial tissues from patients with RA[14]. In murine collagen-induced arthritis, OPN was detected in synovial tissues and at sites of osteoclast-mediated bone resorption, where its expression colocalized to sites of αvß3 integrin expression[15].

To date, it is unclear whether OPN is overexpressed in rheumatoid joints using quantitative methodology and unknown whether OPN expression is associated with gene polymorphism or with susceptibility to RA. These issues are particularly important as OPN genetic polymorphism was found to correlate with other autoimmune conditions where proinflammatory cytokines are critical to the disease process[16,17].

We quantitatively examined expression of OPN in mononuclear cells derived from peripheral blood (PBMC) and synovial fluid (SFMC) of patients with RA and healthy individuals; correlated OPN expression with that of Th1 and Th2 cytokines, namely TNF-α, interferon-γ (IFN-γ), and IL-5; and examined possible relationships/mechanisms responsible for overexpression of OPN gene in the rheumatoid joint, including OPN genetic polymorphisms and the role of inflammatory factors produced in RA joints. To this end, single nucleotide polymorphism (SNP) genetic polymorphisms were analyzed in specimens from patients with RA to determine whether they correlated with the expression of OPN gene and whether they are associated with susceptibility to RA. In parallel, we investigated whether expression of OPN is associated with the synovial inflammatory environment. Our findings provide new insights into the role of OPN in RA.

MATERIALS AND METHODS

Patients and specimens. For analysis of OPN expression, MC preparations were derived from both SF and paired PB specimens of 23 patients with RA (age 54 ± 19 years; disease duration 15 ± 14 yrs; positive rheumatoid factor: 83%) and from blood of healthy individuals (n = 30). Both PBMC and SFMC obtained by SF aspiration were isolated by conventional Ficoll-Paque method and were immediately processed for RNA extraction. For analysis of OPN genetic polymorphism, PBMC specimens were obtained from both RA patients (n = 192) and healthy individuals (n = 288).

Informed consent was obtained from all study subjects. The study was approved by the institutional review board. Differences in sex ratio and age between the RA group and the control group were not significant ($p > 0.05$). All patients fulfilled the American College of Rheumatology criteria for RA[18].

RNA isolation and cDNA synthesis. Total RNA was isolated from cell pellets using RNeasy Mini Kit (Qiagen, Hilden, Germany). Genomic DNA was removed from total RNA prior to cDNA synthesis using the RNase-free DNase Set for DNase digestion during RNA purification (Qiagen). RNA was stored at –80°C. First-strand cDNA synthesis was performed for each RNA sample using Sensiscript RT Kit (Qiagen). Random hexamers were used to prime cDNA synthesis.

Real-time reverse transcription-polymerase chain reaction (PCR) analysis of gene expression of OPN and cytokines. Gene expression of OPN and cytokine mRNA was performed by real-time PCR using specific primers and probes (Table 1). TaqMan Universal PCR master mix (Applied Biosystems, Foster City, CA, USA) was used to measure abundance of PCR products. Thermocycler conditions comprised an initial holding at 50°C for 2 min, then 95°C for 10 min. This was followed by a 2-step PCR program consisting of 95°C for 15 s, and 60°C for 60 s for 40 cycles. Data were collected and quantitatively analyzed on an ABI Prism 7900 sequence detection system (Applied Biosystems). Standard curves were generated and transcript values were calculated as described in User Bulletin #2 ABI Sequence Detection System (Applied Biosystems). The hypoxanthine ribosyltransferase (HPRT) gene was used as an endogenous control to normalize for differences in the amount of total RNA in each sample. TaqMan HPRT gene primers and a 5'-VIC-labeled minor groove binder probe were used according to the manufacturer's instructions (Applied Biosystems). All quantities were expressed as the number of folds relative to the expression of HPRT as a calibrator.

Analysis of OPN polymorphisms. Genomic DNA was extracted from PBC cells using FlexiGene DNA kit (Qiagen). Two SNP sites in the promoter region and 2 SNP in the coding region, along with 2 from noncoding regions of the OPN gene, were analyzed using specific primers. The primer sequences and SNP positions are listed in Table 2. Among the 6 SNP, SNP5 was detected by applying mismatch PCR. A 206-bp fragment was yielded, which introduced an XspI restriction site. SNP4 and SNP6 were detected by standard restriction fragment length polymorphism (RFLP)-PCR. A 290-bp fragment corresponding to SNP4 site was generated, which was recognized by restriction enzyme Alu I. A 568-bp fragment for SNP6 was recognized by restriction enzyme AccI. PCR was performed in a total volume of 10 µl, containing 10 ng of genomic DNA, 2 pmol of each primer, 250 µM dNTP, 10 mM KCl, 20 mM Tris–HCl, 1.5 mM $MgCl_2$, and 0.05 U Taq polymerase. The PCR reaction mixtures were then denatured at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing for 30 s, extension at 72°C for 1 min, and final elongation at 72°C for 7 min. Annealing temperature was set at 55°C for SNP5, 57°C for SNP4, and 58°C for SNP6. After digesting the PCR products with 5 U of either Sau3AI (Takara), Alu I (Takara), or XspI (Takara) for 3 h at 37°C, alleles of each polymorphic site were determined using 3% agarose gel electrophoresis.

As for SNP1, SNP2, SNP3, polymorphisms were detected by direct sequencing. PCR amplification was performed under the following conditions: 94°C for 4 min, 30 cycles; 94°C for 30 s; 55°C for 30 s; and 72°C for 50 s. Five microliters PCR product was incubated with 1 µl (2 U) shrimp alkaline phosphatase and 1 µl exonuclease I at 37°C for 15 min. Then the temperature was increased to 80°C for 15 min. After the purification, the PCR products were subjected to cycle sequencing with the appropriate primers.

Stimulation of OPN expression by SF. PBMC from RA patients and healthy individuals were cultured in 24-well plates at 1 million cells per well in RPMI 1640 medium containing 20% fetal bovine serum in the presence or absence of SF prefiltered through a Millex syringe driven filter unit (0.22 µm; Millipore Corporation) at the indicated dilutions. Cells were maintained at 37°C 5% CO_2 for 48 h and were then harvested for RNA extraction and real-time PCR analysis.

Statistical analysis. Differences in the expression of OPN gene between the groups were analyzed by the Mann-Whitney U test. Correlation between the genotypes of OPN polymorphisms and the onset age of RA was tested by analysis of variance. Statistical differences in the frequencies of various alleles between the groups were tested using the chi-square test for 2-by-2

Table 1. Primer and probe sequences for real-time PCR analysis.

Gene	Primer Sequence	Probe Sequence
OPN	Forward 5'-CTCAGGCCAGTTGCAGCC-3'	FAM 5'-AAACGCCGACCAAGGAAAACTCAC
	Reverse 5'-CAAAAGCAAATCACTGCAATTCTC-3'	TACC-3'MGB
IL-5	Forward 5'-ATGCCATCCCCACAGAAATTC-3'	FAM 5'-AGTGCATTGGTGAAAGAGACCTTGG
	Reverse 5'-CCTCAGAGTCTCATTGGCTATCAG-3'	CACT-3'TAMRA
TNF-α	Forward 5'-TCTTCTCGAACCCCGAGTGA-3'	FAM 5'-TAGCCCATGTTGTAGCAAACCCTCA
	Reverse 5'-CCTCTGATGGCACCACCAG-3'	AGCT-3'TAMRA
IFN-γ	Forward 5'-TCAGCTCTGCATCGTTTTGG-3'	FAM 5'-TTGGCTGTTACTGCCAGGACCCAT
	Reverse 5'-GTTCCATTATCCGCTACATCTGAA-3'	ATGT-3'TAMRA

Table 2. SNP detection methods and nucleotide sequences of the primers.

SNP	NCBI-ID	Position	Variation	Primers	Detection Method
1	Newly identified	–631*	G/T	F 5' GGATGAGGGAACAAGGATAGG 3' R 5' CCAGGAAGAGCACTTAGGGA 3'	Direct sequencing
2	Newly identified	–458*	T/C	F 5' GGATGAGGGAACAAGGATAGG 3' R 5' CCAGGAAGAGCACTTAGGGA 3'	Direct sequencing
3	rs4754	Exon 6	C/T	F 5' CTAATGTGCTATAAAGGCTAAGGG 3' R 5' GGATGTCAGGTCTGCGAAACT 3'	Direct sequencing
4	rs1126616	Exon 7	T/C	F 5' CCGTGGGAAGGACAGTTATG R 5' TTTAATTGACCTCAGAAGATGCAC	PCR-RFLP
5	rs1126772	Exon 7	A/G	F 5' GCATCTTCTGAGGTCAATTAAAAGG R 5' CAGGGAGTTTCCATGAAGCCACAAACTAAACTAATTATCAAACACAC	Mismatch PCR-RFLP
6	rs9138	Exon 7	C/A	F 5' TGGTTGTAGACCCCAAAAGTA R 5' AACCGTGGGAAAACAAATAA	PCR-RFLP

* SNP newly identified in the promoter region and expressed as –631 and –458 from the first base before the first exon according to Genbank data (Locus ID 6696).

or 2-by-3 comparisons. A statistically significant difference was indicated by p values less than 0.05.

RESULTS

Increased expression of OPN mRNA in mononuclear cells of RA patients and its association with the expression of Th1 and Th2 cytokines. SFMC from patients and PBMC from both patients and healthy individuals were analyzed for the expression of OPN mRNA by real-time PCR. As illustrated in Figure 1, mRNA expression of OPN was significantly increased in PBMC derived from RA as compared to that of healthy individuals ($p < 0.05$). mRNA for OPN was undetectable in a larger proportion of healthy controls than in RA patients (53% in controls vs 17% in RA specimens). The expression of OPN mRNA was increased by at least 3 logs in paired SFMC specimens derived from the same RA patients (Figure 1).

Further characterization was performed to examine whether expression of OPN potentially correlated with that of Th1 and Th2 cytokines. To this end, the level of mRNA expression of TNF-α, IFN-γ, and IL-5 was analyzed in the same mononuclear cell specimens by real-time PCR using cytokine-specific primers. As shown in Figure 2, there was significantly higher expression of both TNF-α and IFN-γ in SFMC from RA, and the level of TNF-α remained elevated in PBMC derived from RA patients compared to control

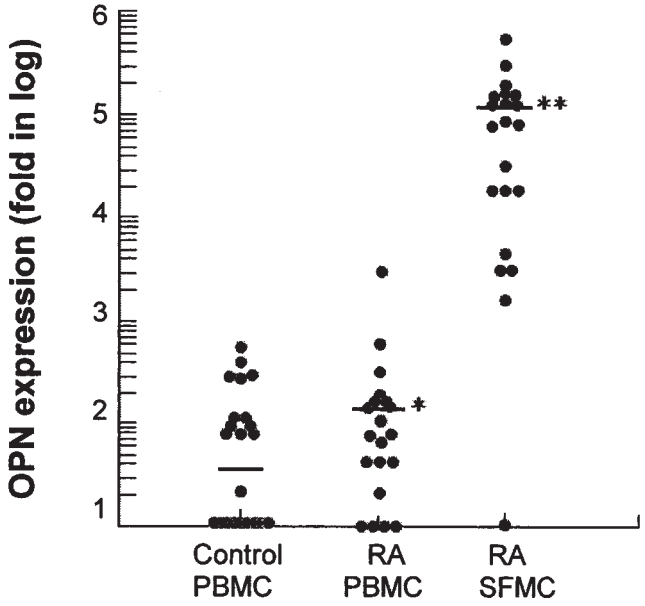

Figure 1. Quantitative real-time PCR analysis of the expression of OPN mRNA in mononuclear cell specimens. Results were normalized to endogenously expressed HPRT in the same cell preparations and are presented as folds relative to that of HPRT. Horizontal bars represent means of OPN expression. *$p < 0.05$; **$p < 0.01$ between the groups. PBMC: peripheral blood mononuclear cells; SFMC: synovial fluid mononuclear cells.

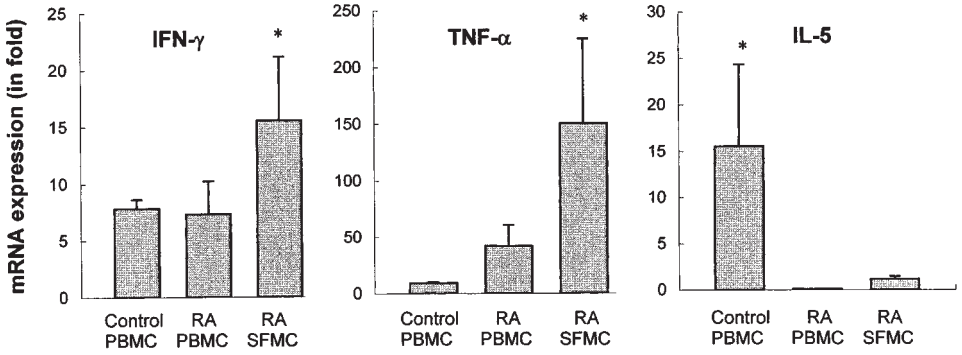

Figure 2. Quantitative real-time PCR analysis of mRNA expression of selected cytokines. Expression of selected cytokine mRNA was normalized to endogenously expressed HPRT in the same cell preparations. *p < 0.05 between groups. PBMC: peripheral blood mononuclear cells; SFMC: synovial fluid mononuclear cells.

PBMC ($p < 0.05$). In contrast, the expression of IL-5 mRNA, a Th2 cytokine, was extremely low in both SFMC and PBMC of RA patients compared to control PBMC ($p < 0.01$). Although the analysis did not reveal statistical significance, owing to the small sample size, there was a trend for positive correlation of TNF-α ($r = 0.40$, $p = 0.12$) and IFN-γ ($r = 0.29$, $p = 0.20$) and negative correlation of IL-5 ($r = –0.32$, $p = 0.17$) with the expression of OPN.

OPN genetic polymorphisms and their potential association with susceptibility to RA. We then addressed whether genetic polymorphisms of the OPN gene were associated with susceptibility to RA. The number of patients and healthy controls were analyzed; their clinical/demographic characteristics are shown in Table 3. It has been reported that there are SNP within the OPN gene and that some of these SNP are associated with other autoimmune conditions[16,17]. A pilot analysis was performed in this study to sequence the full length of the OPN gene in a cohort of RA patients ($n = 42$); analysis revealed a total of 14 SNP sites, including 2 sites in the promoter region that have not been reported. Six SNP sites were selected for analysis; these included 2 newly identified SNP sites in the promoter region and 4 sites in the exons that have demonstrated association with lupus and multiple sclerosis[16,17]. Genotyping of the selected SNP was performed in 192 patients with RA and 288 healthy individuals (all of Chinese Han nationality) by RFLP-PCR or direct DNA sequencing. Table 4 shows the proportion of OPN genotypes and allele frequencies in both groups. The analysis indicated that the prevalence of genotype and allele frequencies at the selected sites did not differ significantly between the RA patient group and the control group ($p > 0.05$). The data were further stratified to delineate potential relationships between OPN genetic polymorphism and clinical characteristics of RA patients based on age of disease onset (Table 5). In addition, there was no apparent association between the OPN polymorphism and rheumatoid factor or C-reactive protein in this cohort of RA patients. Collectively, the analysis does not support association of genetic polymorphism of OPN and susceptibility to RA.

Analysis of association of OPN genetic polymorphism with expression level of OPN and TNF-α. Further characterization was carried out to address whether genetic polymorphisms of OPN were associated with the increased expression of OPN. As polymorphisms at the 5' promoter region of a gene may have a great impact in transcription regulation, 2 SNP sites were selected for analysis to delineate whether polymorphisms affected the expression of OPN in 106 PBMC specimens of 192 RA patients previously examined for susceptibility. As shown in Figure 3A, the results indicated that in both SNP-1 (GG, GT, and TT subgroups) and SNP-2 (CC, CT, and TT subgroups) no significant association with OPN expression was found in RA patients examined (p value was 0.562 and 0.827, respectively). Similarly, the analysis for potential association of OPN genetic polymorphisms with expression of TNF-α, an important proinflammatory cytokine in RA, yielded the same results (Figure 3B). There was no significant association between the SNP subtypes of OPN and TNF-α with their expression levels ($p = 0.69$ and $p = 0.90$, respectively).

Induction of OPN mRNA expression by SF derived from RA patients. Next, we addressed whether the expression of OPN in PBMC could be induced by SF that contained various inflammatory cytokines. PBMC from both RA patients and healthy individuals were exposed, at the indicated dilutions,

Table 3. Clinical and demographic data of patients and controls.

Group	n	Sex, F/M (%)	Age, yrs	RF Positive, %	Disease Duration, yrs
RA	192	163 (84.9)/29 (15.1)	52.75 ± 12	76.6	6.87 ± 8
Control	288	241 (83.7)/47 (16.3)	48.67 ± 18	—	—

RF: rheumatoid factor.

Table 4. OPN polymorphisms and frequencies in RA patients and control subjects.

SNP	Genotype/ Allele	Frequency in Controls No. per 288 Total Analyzed (%)	Frequency in RA Patients No. per 192 Total Analyzed (%)
1	G/G	110 (38.2)	68 (35.4)
	G/T	146 (50.7)	99 (51.6)
	T/T	32 (11.1)	25 (13.0)
	G	366 (63.5)	235 (61.2)
	T	210 (36.5)	149 (38.8)
2	T/T	121 (42.0)	88 (45.9)
	C/T	123 (42.7)	76 (39.5)
	C/C	44 (15.3)	28 (14.6)
	T	365 (63.4)	252 (65.6)
	C	211 (36.6)	132 (34.4)
3	C/C	146 (50.7)	97 (50.5)
	C/T	122 (42.3)	78 (40.6)
	T/T	20 (7.0)	17 (8.9)
	C	414 (71.9)	272 (70.8)
	T	162 (28.1)	112 (29.2)
4	T/T	144 (50.0)	97 (50.5)
	T/C	118 (41.0)	79 (41.1)
	C/C	26 (9.0)	16 (8.4)
	T	406 (70.5)	273 (71.1)
	C	170 (29.5)	111 (28.9)
5	A/A	150 (52.1)	104 (54.2)
	A/G	110 (38.2)	63 (32.8)
	G/G	28 (9.7)	25 (13.0)
	A	410 (71.2)	271 (70.6)
	G	166 (28.8)	113 (29.4)
6	C/C	142 (49.3)	96 (50.0)
	C/A	122 (42.4)	77 (40.1)
	A/A	24 (8.3)	19 (9.9)
	C	406 (70.5)	269 (70.1)
	A	170 (29.5)	115 (29.9)

Table 5. Age of disease onset and the OPN genotypes.

SNP	Genotype	Age of Onset, yrs (mean ± SD)	No. per 192 RA Analyzed
1	G/G	45.4 ± 14.3	68
	G/T	46.9 ± 11.5	99
	T/T	43.4 ± 9.5	25
2	T/T	45.6 ± 12.0	88
	C/T	47.3 ± 12.2	76
	C/C	43.3 ± 13.5	28
3	C/C	45.5 ± 11.8	97
	C/T	46.9 ± 13.1	78
	T/T	44.2 ± 12.6	17
4	T/T	45.2 ± 11.6	97
	T/C	47.3 ± 13.2	79
	C/C	43.2 ± 12.5	16
5	A/A	45.6 ± 12.1	104
	A/G	46.5 ± 12.2	63
	G/G	45.8 ± 14.2	25
6	C/C	45.1 ± 11.8	96
	C/A	46.9 ± 12.8	77
	A/A	46.1 ± 13.8	19

to RA SF and analyzed for mRNA expression of OPN using real-time PCR. As shown in Figure 4, OPN expression was significantly elevated in PBMC in the presence of RA SF. The stimulating effect of SF on the expression of OPN was potent but not specific for RA patients, as it had the same effect on control PBMC (Figure 4A), and the observed effect was dose-dependent (Figure 4B). The results indicate that OPN expression could be induced by SF that represents the rheumatoid synovial environment.

DISCUSSION

The etiology and pathogenesis of RA remains unclear. There are multiple factors — environmental and genetic, as well as dysregulation of the immune system — that have been implicated in the pathogenesis of RA and synovial pathology[19,20]. Additional proinflammatory mediators further contribute to and complicate the perpetuation of inflammation in the RA synovium. The importance of proinflammatory mediators/cytokines is supported by reasonable efficacy for the treatment of RA using agents to antagonize TNF-α or its receptors[21]. Further characterization of the role of new proinflammatory mediators/cytokines and the molecular mechanism for their activation and interaction is a current focus in RA research.

OPN has recently been recognized as an important proinflammatory cytokine potentially involved in joint inflammation. Among its multiple properties, OPN has been shown to act as a Th1 cytokine and has been implicated in the inflammatory responses through recruitment of inflammatory cells and augmentation of cytokine expression, including TNF-α and integrins[22]. Our own study to delineate differential expression of synovial tissue antigens in RA compared to controls has revealed predominant overexpression of the OPN gene in RA synovial lesion tissues by cDNA microarray technology (manuscript in preparation).

In the current study, we demonstrate increased transcription of the OPN gene in PBMC and SFMC derived from RA patients compared to those of healthy controls. Our finding is consistent with a recent report by Petrow and colleagues, who described increased expression of OPN in RA patient SF[14]. A major focus of our current study was to address possible mechanisms potentially responsible for the overexpression of the OPN gene in mononuclear cells in RA.

Two possibilities were examined. First, we investigated whether the overexpression of OPN gene was associated with its genetic polymorphisms and whether polymorphisms of OPN gene contributed to susceptibility to RA. There is some preliminary evidence suggestive of such an association with susceptibility to autoimmune conditions. Recently, Forton, *et al*[16] reported that SNP4 (the 9250th position) was significantly associated with SLE. In another report, Iwasaki and co-workers[17] found significant association of age of disease onset in multiple sclerosis (MS) with SNP5 (position 9583), and susceptibility to MS with SNP3 (position 8090).

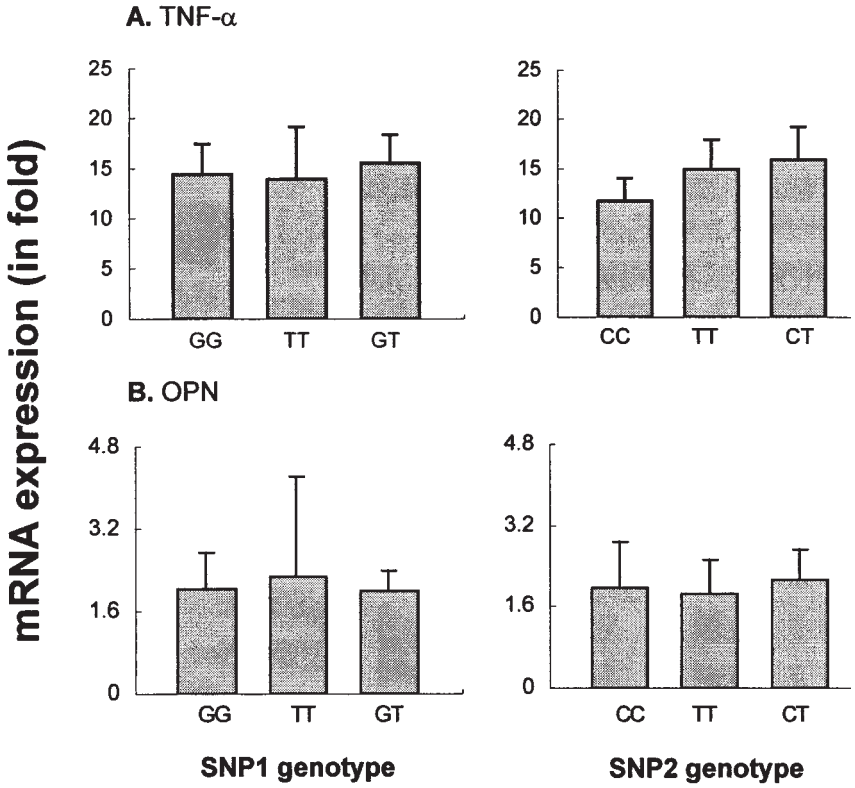

Figure 3. Correlative analysis of 2 SNP sites in the promoter region with the expression of OPN and TNF-α in peripheral blood mononuclear cells (PBMC). Genotypes of SNP1 (–631) and SNP2 (–458) were analyzed in association with the expression of OPN (panel A) and TNF-α (Panel B) in PBMC specimens. The genotypes analyzed were GG (n = 40), TT (n = 12), and GT (n = 54) for SNP1; and CC (n = 15), TT (n = 46), and CT (n = 45) for SNP2.

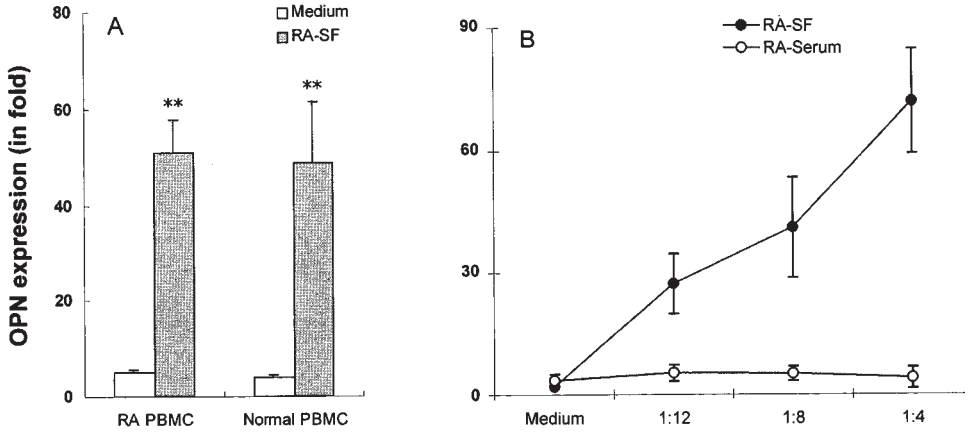

Figure 4. Quantitative real-time PCR analysis of OPN mRNA expression in synovial mononuclear cells after treatment with RA synovial fluid (SF). A. Prefiltered SF of a patient with RA was added, at a final dilution of 1:5, to PBMC culture derived from RA patients (RA-PBMC, n = 6) or healthy individuals (normal PBMC, n = 10). After 48-h incubation, cells were collected for real-time PCR analysis of OPN mRNA expression. The same experiment was repeated with 2 other SF samples from different patients with similar results. B. Dose-response pattern of OPN expression in response to the indicated dilutions of SF under the same experimental conditions as described in A. **$p < 0.01$, SF versus control medium.

However, it should be noted that a followup study in a large cohort of 1056 patients with MS arrived at a different conclusion that could not confirm association of the OPN genetic polymorphism with susceptibility to MS[23]. To our knowledge, ours is the first study in RA to address association of genetic polymorphisms of the OPN gene with susceptibility to RA and with the expression of OPN. We, however, found no evidence for association of the 6 OPN genetic polymorphisms studied with disease susceptibility or with age of disease onset of RA. Our further characterization showed that OPN genetic polymorphisms analyzed had no significant association with the increased OPN transcription found in mononuclear cells derived from RA patients, nor with expression of TNF-α. Hence, the results described here suggest an alternative possibility: increased expression of OPN gene may be attributable to proinflammatory mediators and cytokines produced abundantly in the inflamed joint. Our observation that the expression of OPN gene was increased by at least 3 logs in SFMC as compared to PBMC in RA patients supports this possibility.

To evaluate the alternative possibility, we examined the potential inducing effect of SF obtained from RA on mRNA expression of OPN. Our results have demonstrated that SF derived from RA patients had a potent stimulating effect on mRNA expression of the OPN gene in mononuclear cells, supporting the possibility that proinflammatory factors produced in the rheumatoid SF are responsible for the overexpression of OPN. As specimens of the other forms of arthritis were not available for analysis in this study, it is not clear whether the observed overexpression of OPN in SFMC is specific for RA and whether the stimulating effect of SF on the OPN gene is associated with RA. Further, as many proinflammatory cytokines and chemokines are produced at high concentrations in RA SF, it is conceivable that some of these cytokines and chemokines, such as TNF-α and IFN-γ, may be attributable to the upregulation of OPN expression as a mediator. Systemic investigation is under way in our laboratory to define the role of individual inflammatory factor(s) produced in rheumatoid synovitis in the activation of signaling events leading to overexpression of the OPN gene.

ACKNOWLEDGMENT

We thank Yan Dong and Yayun Shen for their help in DNA sequencing and Yingyun Zhang for technical support.

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