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Domain reactivity of autoantibodies to calpastatin in patients with systemic rheumatic diseases

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Abstract Autoantibodies to calpastatin (endogenous inhibitor of calpain, a calcium-dependent neutral proteinase) have been detected in sera of patients with rheumatoid arthritis (RA) and other diseases. We investigated the epitope reactivity of anticalpastatin autoantibodies in patients with rheumatic diseases. cDNAs encoding each calpastatin domain (L, I, II, III, and IV) were amplified by PCR and ligated into an expression vector. The fusion proteins were expressed in *E. coli*. The presence of autoantibodies specific for each calpastatin domain was assayed in sera of patients with various rheumatic diseases by immunoblotting the fusion proteins with these sera. Of the RA patient sera, 81% reacted with at least one calpastatin domain. This reaction was significantly greater than with sera from patients with systemic lupus erythematosus (46%), scleroderma (32%), polymyositis/dermatomyositis (43%), and normal controls (13%). Domains I and II were recognized by RA patient sera significantly more than by other patient sera, whereas domains III and IV reacted almost equally among all patient sera. Although, collectively, sera from RA and lupus patients reacted equally with all domains, scleroderma sera tended to react with only domains I and IV and myositis sera tended to recognize only domains III and IV. Patients with RA positive for anticalpastatin antibodies exhibited more active disease (i.e., a higher erythrocyte sedimentation rate and C-reative protein level) than antibody-negative patients. Our results suggest that anticalpastatin antibodies were detected in RA with the highest frequency and that different domain reactivity was shown among different diseases. The presence of these antibodies in sera may be related to the type of disease and, in RA, with disease activity, suggesting their importance in rheumatic disorders.

Key words Autoantibody · Calpastatin · Calpain · Rheumatoid arthritis · Autoimmune disease

Introduction

Rheumatoid arthritis (RA), which is characterized by the presence of chronic polyarthritis and joint destruction, is included among systemic autoimmune diseases because of the characteristic presence of rheumatoid factor (autoantibodies to the Fc portion of IgG). Other autoantibodies specific for RA rarely have been described. We have previously shown that sera from patients with RA contain a variety of autoantibodies to unknown proteins.[1] By molecular cloning of the target antigens, we and other investigators have demonstrated that calpastatin is one of such autoantigens. This substance is an endogenous protein inhibitor of calpain (EC 3.4.22.17), a calcium-dependent neutral proteinase.[2,3] Calpastatin and calpain are widely distributed in the cytoplasmic fraction of nearly all mammalian cells. Human calpastatin contains 673 amino acid residues, and its estimated molecular weight is 72 605.[4] The calpastatin molecule consists of an N-terminal domain (domain L) and four repetitive domains (domain I, II, III, and IV). Each of the latter four domains binds to and inhibits one calpain molecule, and each contains a consensus sequence of seven amino acids (TIPPXYR), thought to be the binding site to calpain.[5] Although calpain is known to degrade a variety of substrates, including intracellular enzymes, hormone receptors, and various membrane and cytoskeletal proteins,[6] the physiological and pathophysiological roles of calpain and calpastatin are not well understood. Several reports suggest that calpain may participate in various inflammatory processes, through protein kinase C activation,[7,8] exocytosis of granules and superoxide production in neutrophils,[9] secretion and activation of

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interleukin-1α,[10] and generation of chemotactic factor by autodigestion.[11] Moreover, levels of calpain are increased in the synovial fluid of patients with arthritis,[12–14] and, at least in vitro, calpain degrades cartilage proteoglycan.[15] Taken together, these findings indicate that anticalpastatin autoantibodies may increase the enzymatic activity of calpain, thus enhancing inflammation and joint destruction.[16] As the IgG fraction of RA sera that contains anticalpastatin antibodies has been shown to block calpastatin-mediated inhibition of calpain protease activity,[2,17] this suggests that, in this disease, the presence of autoantibodies in the serum may enhance the relative activity of calpain and may play an important role in the pathology of RA.

We have previously analyzed the reactivity of these autoantibodies to only a part of the calpastatin molecule (domain IV and part of domain III). It is therefore unclear if autoantibodies in patient sera can react with the remainder of the calpastatin molecule. To determine this, we investigated the reactivity of these autoantibodies with each calpastatin domain expressed from the full-length cDNA encoding human calpastatin, and we determined the clinical significance of each set of autoantibodies in rheumatic disorders.

Materials and methods

Sera

We obtained sera from 47 Japanese patients who fulfilled the revised criteria for RA of the American Rheumatism Association.[18] Sera were obtained from 28 patients with systemic lupus erythematosus (SLE),[19] 25 with systemic sclerosis (SSc),[20] and 21 patients with polymyositis/dermatomyositis (PM/DM).[21] Thirty sera from healthy volunteers were used as normal controls.

Cloning of the full-length cDNA encoding human calpastatin

Using the 1.2-kb partial cDNA (RA-6) encoding the C-terminal 178 amino acids of human calpastatin[2] as a probe, λgt11 cDNA libraries, constructed from human placental mRNA (Clontech, Palo Alto, CA, USA) and human thyroid mRNA (constructed by random primers; gift from Dr. T. Yoshida, Keio University), were screened by plaque hybridization technique using an ECL kit (Amersham, Buckinghamshire, England).[22] Positive plaques were picked up and isolated by repeated screening until all progeny plaques were positive. cDNAs were purified from isolated λ phages containing positive clones. Oligonucleotide primers (28 nucleotides) encoding the 5′-terminal sequences with *Eco*RI linkers, and the 3′-terminal sequences with *Pst*I linkers, of calpastatin domains L, I, II, and III were synthesized (Table 1).[4] For domain IV, the 3′-reverse primer was attached with a *Sal*I linker because this domain contained a *Pst*I site (Table 1). All these primers were designed so that the reading frames of the products would align with cro-β-galactosidase when subcloned into the *Eco*RI site of pEX-2 plasmid DNA. cDNAs encoding each calpastatin domain were amplified by the polymerase chain reaction (PCR) using these primers, Taq polymerase (Takara Shuzo, Kyoto, Japan), and the respective λ phage cDNAs as templates. Some of the PCR products were fractionated on 1% agarose gels to confirm amplification.

Subcloning of cDNA into an expression vector and expression of fusion proteins

Subcloning of cDNA

PCR products were digested with *Eco*RI and *Pst*I (or *Sal*I for domain IV) for 60 min at 37°C, and 100 ng of each was ligated into 20 ng pEX-2 plasmid DNA (Boehringer-Mannheim, Mannheim, Germany), which had been digested with *Eco*RI and *Pst*I (or *Sal*I for domain IV), for 12 h at 15°C. Ligated cDNAs were incubated with $CaCl_2$-competent *E. coli* POP2136, spread on a Luria-Bertani (LB) agar plate (90-mm diameter) containing ampicillin (50 mg/ml), and cultured overnight at 32°C. Colonies were transferred to two nitrocellulose (NC) filters (81-mm diameter; BA-85; Schleicher & Schuell, Dassel, Germany). Each replicate filter was plated on a new agar plate with colonies up, and cultured again for 12 h at 32°C.

Colony hybridization

One NC filter with cultured colonies was treated serially with (1) 0.5 M NaOH, 1.5 M NaCl, (2) 0.5 M Tris-HCl, 1.5 M NaCl, pH 7.4, and (3) 30 mM sodium citrate, 0.3 M NaCl for 5 min each, and baked for 2 h at 80°C. This filter was

Table 1. Primers for PCR amplification of human calpastatin domains

Domain (position)	5′-primer	3′-primer
L (1–392)	P1: CCGAATTCATGAATCCCACAGAAACCAA	P2: GGCTGCAGATGCCTGACTTTCCCGATGG
I (367–756)	P3: CCGAATTCGATAAACCATCGGGAAAGTC	P4: GGCTGCAGACTCATTGTATCCTTCTTCA
II (730–1202)	P5: CCGAATTCAGAAAGGTGGAGAAGGATAC	P6: GGCTGCAGGCTTCTACAGCACCCCCTGG
III (1138–1595)	P7: CCGAATTCATACAGTCAGCACCCCCTGA	P8: GGCTGCAGAGGTCTTTGTCACTCTGCGA
IV (1570–2019)	P9: CCGAATTCGATACCTCGCAGAGTGACAA	P10: GGGTCGACGTCATCTTTTGGCTTGGAAG

The linker sequences are underlined

screened by hybridization for 12 h at 42°C with peroxidase-labeled PCR-cDNAs (ECL kit; Amersham).

Colony blotting

A second NC filter with cultured colonies was further cultured for 2 h at 42°C to express fusion proteins of cDNA products and cro-β-galactosidase. This filter was dipped into a lysozyme solution (8 mg/ml in 25 mM Tris-HCl, 50 mM glucose, 10 mM EDTA, pH 8.0) for 5 min to lyse bacteria, and blocked for 1 h with 2% (w/v) skim milk (Morinaga Nyugyo, Tokyo, Japan) in TBS (10 mM Tris-HCl, 0.15 M NaCl, pH 7.5). The filter was incubated with mouse monoclonal antihuman calpastatin antibodies (each 1:4000) specific for each domain [CSF1-2 (domain I), CSF3-3 (domains II + IV), and CSL1-5 (domain III); Takara Shuzo], and then with alkaline phosphatase-conjugated goat antimouse IgG antibody (1:7500; Promega, Madison, WI, USA). Positive colonies, expressing calpastatin fusion proteins, were detected by reaction with nitroblue tetrazolium (NBT) and 5-bromo-3-chloro-indolyl-phosphate (BCIP).

Confirmation of expression of fusion proteins by immunoblotting

Clones positive by both colony hybridization and colony blotting were picked and cultured in 20 ml LB medium containing ampicillin for 12 h at 32°C. After incubation for 2 h at 42°C, bacteria expressing fusion proteins were obtained by centrifugation, dissolved in 1 ml SDS-sample buffer (62.5 mM Tris-HCl, 0.01% bromophenol blue, 5% 2-mercaptoethanol, 1% SDS, 10% glycerol, pH 6.8), boiled for 5 min, and fractionated on an SDS-polyacrylamide gel (5%–15% gradient gel). Proteins were transferred electrophoretically to a NC filter (BA-85; Schleicher & Schuell).[23] After blocking with 2% skim milk/TBS for 1 h at room temperature, the filter was incubated with mouse monoclonal antibodies (CSL1-5, CSF3-3, and CSF1-2) and with alkaline phosphatase-conjugated goat antimouse IgG antibody, and the fusion proteins were detected by the NBT/BCIP reaction.

Reaction of patient sera with calpastatin domain fusion proteins

Fusion proteins expressed from clones encoding each calpastatin domain were fractionated on SDS-7.5% polyacrylamide gels and transferred electrophoretically to NC filters. After blocking with 2% skim milk for 1 h at room temperature, the filters were cut into 4-mm strips, each of which was incubated with 2 ml of a 1:100 diluted patient serum (preabsorbed with *E. coli* lysates) for 2 h at room temperature. After washing three times with TBS containing 0.05% Nonidet P-40, the filter strips were incubated with alkaline phosphatase-conjugated goat antihuman IgG (Promega; 1:7500) for 1 h at room temperature, and reactivity was detected by the NBT/BCIP reaction.

Results

Cloning of cDNAs encoding human calpastatin

Using the RA-6 partial cDNA probe, 5×10^5 clones were screened, and 13 positive clones were isolated. Using synthesized primers (Table 1) and DNAs from these clones as templates, cDNA for each calpastatin domain (L, I, II, III, and IV) was amplified by PCR. In only 1 clone (termed KSK-26), sequences for domains L, I, and II were amplified. The other 12 clones were found to cover domain II, III, and/or IV sequences. Therefore, the KSK-26 clone was used to generate domain L, I, and II cDNAs, whereas domains III and IV cDNAs were generated from another clone termed KSK-17 that was found to cover domain II, III, and IV sequences (Fig. 1). These cDNAs were subcloned into M13 mp18 or M13 mp19 RF DNA to ascertain their amino acid sequences. For domains I, II, III, and IV, the sequences were exactly as previously described,[4] except for one amino

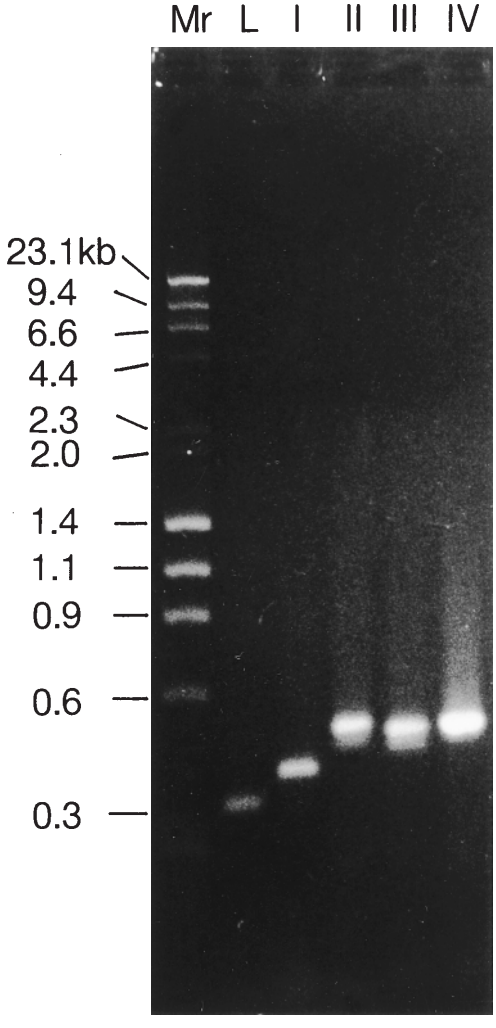

Fig. 1. PCR-amplified calpastatin domains cDNAs. cDNA sequences for each domain were amplified by PCR from cloned calpastatin cDNA and fractionated on 1% agarose gel. The domain L, I, and II cDNAs were amplified from clone KSK-26, and the domain III and IV cDNAs were amplified from clone KSK-17

acid substitution (G to E at position 557) in domain IV.[2] However, our subcloned domain L lacked 57 bp (19 amino acids) of the original sequence and had several amino acid substitutions.[4] This sequence was completely identical with that described by Lee et al., which appeared to be transcribed by exon skipping.[24]

Subcloning of cDNAs into an expression vector and expression of fusion proteins

When we subcloned each calpastatin domain cDNA into the prokaryotic expression vector pEX-2 and assayed the reactivity of the resulting fusion proteins with monoclonal antibodies to human calpastatin, we found that the fusion proteins of domains I, III, and II + IV reacted specifically with the monoclonal antibodies CSF1-2, CSL1-5, and CSF3-3, respectively (Fig. 2). Because domain L did not react with any monoclonal antibody, a positive clone detected by colony hybridization was selected.

Reaction of patient sera with calpastatin domain fusion proteins

We next examined the reactivity of sera from 121 patients with systemic rheumatic diseases and 30 healthy volunteers with each calpastatin domain. The reactivity patterns for calpastatin domains varied among the patient sera (Fig. 3). Domain L was recognized by the sera of only a small number of patients, and no statistical significance was observed among the domain L reactivity of each disease and normal group. Domain I was recognized by 49% of RA sera, and this reaction was significantly greater than for any other diseases (10%–20%) and normal subjects (10%). Likely, domain II reactivity was also significantly higher in RA (32%) than in others (except for SLE; $P < 0.1$). In contrast, domains III and IV were reactive almost equally with RA and other rheumatic diseases (except for domain III with SSc). However, all these domain III and IV reactivities in rheumatic patient sera (except for domain III in SSc) were significantly higher than in normal controls (Table 2).

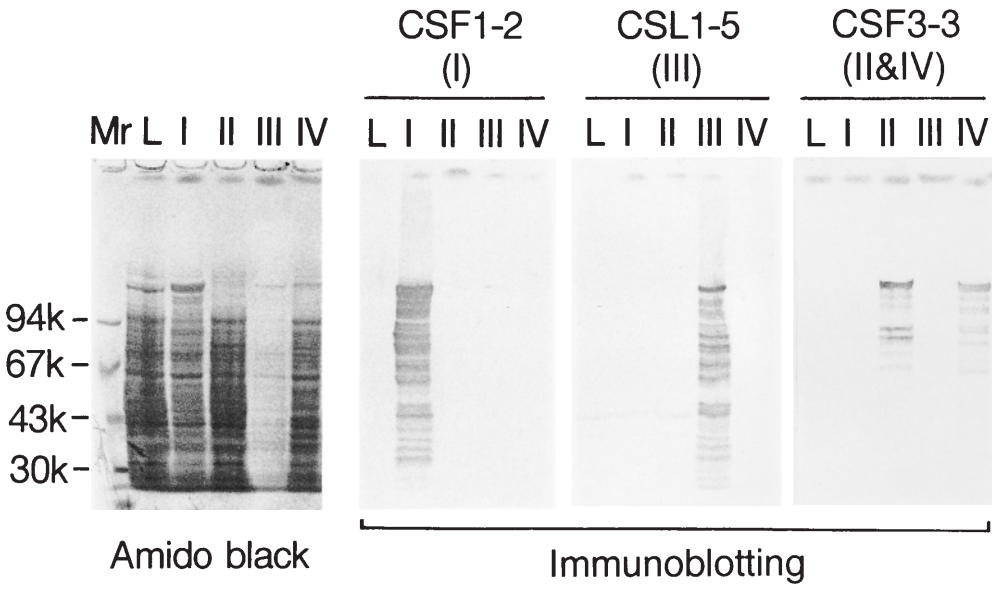

Fig. 2. Expression of fusion proteins for calpastatin domains and immunoblotting with mouse monoclonal anticalpastatin antibodies. *Left panel*, fusion proteins stained with amido black. *Right panel*, immunoblotting of fusion proteins with mouse monoclonal anticalpastatin antibodies CSF1-2 (specific for domain I), CSL1-5 (specific for domain III), and CSF3-3 (specific for domains II and IV). *Smaller bands* represent degradation products of fusion proteins

Table 2. Reactivity to calpastatin domains in sera of patients with systemic rheumatic diseases

Disease	No.	Domain reactivity				
		L	I	II	III	IV
RA	47	5 (11%)	‡23 (49%)	‡15 (32%)	†14 (30%)	†13 (28%)
SLE	28	2 (7%)	4 (14%)†	*4 (14%)	5 (18%)	5 (18%)
SSc	25	0 (0%)	5 (20%)*	2 (8%)*	1 (4%)†	*6 (24%)
PM/DM	21	1 (5%)	2 (10%)†	2 (10%)*	*5 (24%)	*5 (24%)
Normal	30	2 (7%)	3 (10%)‡	0 (0%)‡	1 (3%)†	1 (3%)†

RA, rheumatoid arthritis; SLE, systemic lupus erythematous; SSc, systemic sclerosis; PM/DM, polymyositis/dermatomyositis

*, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$ compared with RA (right shoulder) and normal control (left shoulder) (Fisher's exact test)

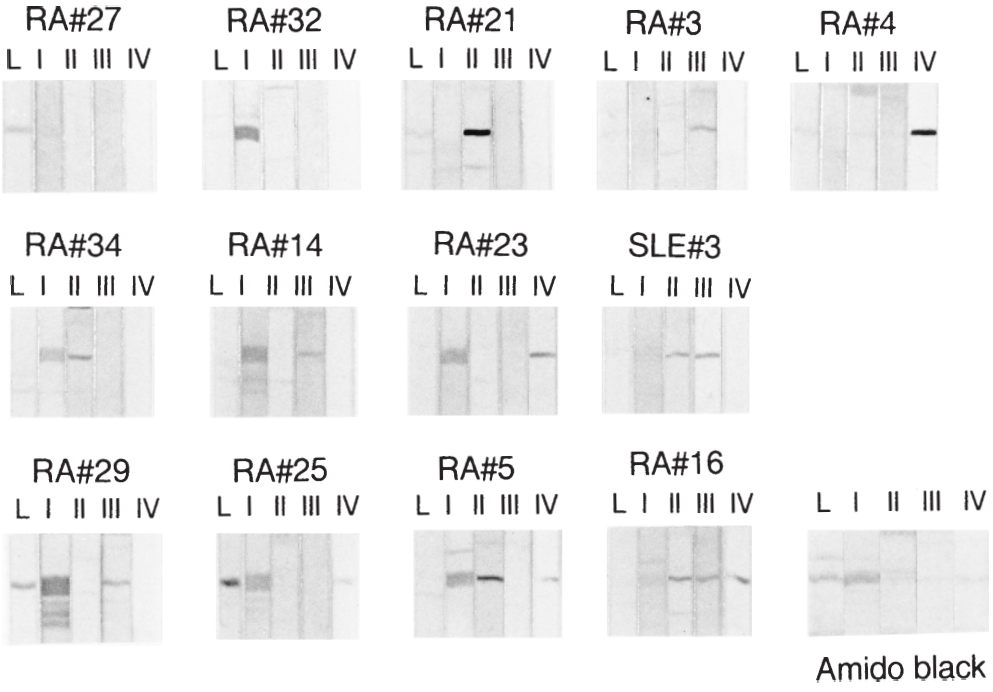

Fig. 3. Reaction of patient sera with calpastatin domain fusion proteins. Reactivities of representative RA and SLE patient sera

When segregated by patient type, it was observed that sera from RA patients reacted with all domains of calpastatin with the highest frequencies. SLE patient sera also reacted with all domains, but the frequencies were lower than with RA sera. SSc sera tended to react mainly with domains I and IV, and PM/DM sera tended to recognize mainly domains III and IV. Even normal sera reacted with all domains except for domain II, although the frequency was much lower than that of patient sera. Sera from two RA patients reacted only with the larger RA-6 fusion protein (containing domain IV and partial domain III) but not with any of the domain fusion proteins (data not shown).

Table 3. Frequency of anticalpastatin antibodies in systemic rheumatic diseases

Disease	No.	RA-6	Domain reactivity		
			≧1	≧2	≧3
RA	47	‡24 (51%)	‡38 (81%)	‡23 (49%)	*7 (15%)
SLE	28	5 (18%)†	‡3 (46%)†	5 (18%)†	2 (7%)
SSc	25	6 (20%)*	8 (32%)‡	5 (20%)*	1 (4%)
PM/DM	21	5 (24%)*	*9 (43%)†	3 (14%)†	2 (9%)
Normal	30	2 (6%)‡	4 (13%)‡	3 (10%)‡	0 (0%)*

*, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$ compared with RA (right shoulder) and normal control (left shoulder) (Fisher's exact test)

Frequency of reactivity to calpastatin domains in systemic rheumatic diseases

When arranged by frequency of reaction with calpastatin domains, we found that 81% of RA sera reacted with at least one domain, whereas significantly lower percentages of sera from SLE, SSc, and PM/DM patients reacted with one domain (Table 3). Almost half (49%) of RA sera and lower percentages of SLE (18%), SSc (20%), and PM/DM (14%) sera reacted with two or more domains, and only a small percentage of patient sera reacted with three or more domains (Table 3). Of the sera that reacted with more than two domains, all three SSc and 19 of 23 RA reacted with domains I and IV.

Clinical correlation of domain reactivity in patients with RA

When we assessed the clinical correlation of domain reactivity of anticalpastatin antibodies in RA, we found that RA patients whose sera reacted with domain I were more likely to be anemic and showed higher erythrocyte sedimentation rate (ESR) than those with sera unreactive with domain I (Table 4; $P < 0.05$ for both). Moreover, RA patients whose sera reacted with at least one calpastatin domain had higher ESR ($P < 0.05$) and C-reactive protein (CRP) ($P < 0.01$) than those with sera unreactive with any domain (Table 4).

Discussion

On cloning the cDNAs corresponding to the target antigens of newly identified autoantibodies in sera from RA patients, we[2] and Despres et al.[3] independently demonstrated that one of these autoantigens is calpastatin, a specific endogenous inhibitor of the calcium-dependent cysteine proteinase, calpain. We detected anticalpastatin antibodies in 57% of RA patients, and in lower percentages of patients with SLE, PM/DM, and SSc, when we used a fusion protein

Table 4. Clinical correlation of domain reactivities of anticalpastatin autoantibodies in RA patients

Clinical manifestation	Domain I		Any domain	
	Positive (n = 23) (%)	Negative (n = 23) (%)	Positive (n = 37) (%)	Negative (n = 9) (%)
Stage ≧2	65	61	65	56
Class ≧2	74	57	68	56
Subcutaneous nodule	14	19	17	14
Sjögren's syndrome	52	35	41	56
Raynaud's phenomenon	14	14	12	22
Pleurisy	9	0	6	0
Pulmonary fibrosis	9	10	8	13
Vasculitis	9	5	6	13
Lymphadenopathy	23	18	20	11
Anemia	52*	22	41	22
Rheumatoid factor	83	74	81	67
Positive ANA	65	52	62	44
ESR (mm/h)	75.2 ± 40.3*	41.9 ± 25.8	63.8 ± 38.5*	36.8 ± 26.5
CRP (mg/dl)	2.99 ± 3.01	1.68 ± 2.20	2.70 ± 2.89†	0.81 ± 0.74

ANA, antinuclear antibodies; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein
*, $P < 0.05$; †, $P < 0.01$ compared with negative patients (Fisher's exact test and Student's t-test)

expressed from a partial cDNA for calpastatin as an antigen. Despres et al., however, detected anticalpastatin antibodies in RA but not in SLE or osteoarthritis patients.[3] This discrepancy may reflect racial differences or differing genetic backgrounds, but more detailed investigation of the calpastatin epitope specificity of these autoantibodies is necessary for this to be determined.

When we amplified the calpastatin domains, we observed that domain L lacked 19 amino acids of the original sequence and had several amino acid substitutions.[4] It has been reported that domain L revealed molecular heterogeneity by exon skipping or alternate splicing.[24] The sequence of our clone was identical with that which lacked exon 3 of domain L as described by Lee et al.[24] Because this clone was isolated from a human thyroid gland cDNA library, it suggests that domain L is diversified among different tissues. Unlike domains I–IV, the function of the domain L is not known. These results, suggesting tissue-specific domain L sequences, raise the possibility that this domain may be involved in expression or localization of calpastatin.

When we assayed the domain reactivity of anticalpastatin antibodies in sera of patients with various rheumatic diseases, we found that these sera showed different degrees of reactivity with the calpastatin domains. This result suggests that each domain has a specific epitope and that anticalpastatin antibodies do not cross-react with multiple calpastatin domains. However, we did note that patient sera reacting with two or more domains tended to react with domains I and IV, suggesting there may be a cross-reactive epitope in these two domains.

In many autoimmune diseases, it is thought that the autoantigen has a major epitope, recognized by almost all patient sera, and several minor epitopes recognized by individual sera.[25–29] However, we found no calpastatin domain with which most patient sera reacted. The most frequent reactivity in RA occurred in only 49% of patients with domain I, and, except for domain L, each domain reacted with similar numbers of sera. The possibility cannot be ruled out, however, that there may be conformational epitope(s) constructed from regions of several domains. Indeed, several sera from RA patients reacted with the RA-6 fusion protein (coding domains III and IV), but not with any of the individual domains of calpastatin. Sera from patients with other autoimmune diseases (SLE, PM/DM, and SSc) had different patterns of calpastatin epitope recognition than did sera from RA patients. The reactivity of anticalpastatin antibodies against domains I and II was significantly higher in RA than in other diseases. In contrast, the reactivity against domains III and IV appeared to be to the same extent among RA and other diseases. These results suggest that anticalpastatin autoantibodies may have different pathological characteristics between RA and other rheumatic diseases. Moreover, the prevalence of anticalpastatin antibodies in RA was significantly higher than in these other systemic rheumatic diseases. It should be noted that 81% of RA sera reacted with at least one or more calpastatin domains. However, anticalpastatin antibodies are not the "specific" marker antibodies of RA, because 32%–46% of sera from other systemic rheumatic diseases also contain antibodies. Nevertheless, the possibility may be raised that anticalpastatin antibodies might serve as a diagnostic marker of RA, because the frequency of these antibodies in RA patients is as high as that of rheumatoid factor. Even some normal control sera appeared to have anticalpastatin antibodies that reacted with several domains. Although the prevalence of such naturally occurring autoantibodies was very low in the normal population, its significance is unknown. These reactions of normal sera appeared to be rather weaker, although immunoblotting is not a suitable method to estimate antibody titer. A quantitative measurement should be necessary to clarify the true significance of such "normal" autoantibodies.

In addition to their possible usefulness in diagnosing RA, our findings concerning anticalpastatin antibodies may have important clinical ramifications. For example, we observed a significantly higher frequency of anemia and a higher ESR in patients whose sera reacted with calpastatin domain I. Because the domain I reactivity was the highest in RA patients, this correlation appears to be especially important. Moreover, RA patients whose sera reacted with any calpastatin domain had significantly higher ESR and CRP levels than patients whose sera was unreactive. The results indicate that the presence of anticalpastatin antibodies in the sera of RA patients is correlated with disease activity. These findings support the hypothesis that these autoantibodies may be closely associated with the development of rheumatic disorders.

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References

1. Nojima T, Kamata M, Matsunobu T, et al. Detection of autoantibodies in sera from patients with rheumatoid arthritis. Jpn J Rheumatol 1994;6:189–200.
2. Mimori T, Suganuma K, Tanami Y, et al. Autoantibodies to calpastatin (an endogenous inhibitor for calcium-dependent neutral protease, calpain) in systemic rheumatic diseases. Proc Natl Acad Sci USA 1995;92:7267–71.
3. Despres N, Talbot G, Plouffe B, et al. Detection and expression of a cDNA clone that encodes a polypeptide containing two inhibitory domains of human calpastatin and its recognition by rheumatoid arthritis sera. J Clin Invest 1995;95:1891–6.
4. Asada K, Ishino Y, Shimada M, et al. cDNA cloning of human calpastatin. Sequence homology among human, pig, and rabbit calpastatins. J Enzyme Inhibition 1989;3:49–56.
5. Emori Y, Kawasaki H, Imajoh H, et al. Endogenous inhibitor for calcium-dependent cysteine protease contains four internal repeats that could be responsible for its multiple reactive sites. Proc Natl Acad Sci USA 1987;84:3590–4.
6. Murachi T. Calpain and calpastatin. Trends Biochem Sci 1983;8: 167–9.
7. Inoue M, Kishimoto A, Takai Y, et al. Studies on a cyclic nucleotide-independent protein kinase and its proenzyme in mammalian tissues. II. Proenzyme and its activation by calcium-dependent protease from rat brain. J Biol Chem 1997;252:7610–6.
8. Kishimoto A, Mikawa K, Hashimoto K, et al. Limited proteolysis of protein kinase C subspecies by calcium-dependent neutral protease (calpain). J Biol Chem 1989;264:4088–92.
9. Pontremoli S, Melloni E, Damiani G, et al. Effects of a monoclonal anti-calpain antibody on responses of stimulated human neutrophils. J Biol Chem 1988;263:1915–9.
10. Kobayashi Y, Yamamoto K, Saido T, et al. Identification of calcium-activated neutral protease as a processing enzyme of human interleukin 1 alpha. Proc Natl Acad Sci USA 1990;87:5548–52.
11. Kunimatsu M, Higashiyama S, Sato K, et al. Calcium dependent cysteine proteinase is a precursor of a chemotactic factor for neutrophils. Biochem Biophys Res Commum 1989;164:875–82.
12. Fukui I, Tanaka K, Murachi K. Extracellular appearance of calpain and calpastatin in the synovial fluid of the knee joint. Biochem Biophys Res Commun 1989;162:559–66.
13. Suzuki K, Shimizu K, Hamamoto T, et al. Biochemical demonstration of calpains and calpastatin in osteoarthritic synovial fluid. Arthritis Rheum 1990;33:728–32.
14. Yamamoto S, Shimizu K, Shimizu K, et al. Calcium-dependent cysteine proteinase (calpain) in human arthritic synovial joints. Arthritis Rheum 1992;35:1309–17.
15. Suzuki K, Shimizu K, Hatamoto T, et al. Characterization of proteoglycan degradation by calpain. Biochem J 1992;285:857–62.
16. Menard HA, El-Amine M. The calpain-calpastatin system in rheumatoid arthritis. Immunol Today 1996;17:545–7.
17. Mimori T, Suganuma K, Tanami Y, et al. A pathogenic role of autoantibodies to calpastatin in patients with rheumatoid arthritis (abstract). Arthritis Rheum 1995;38:S165.
18. Arnett FC, Edworthy S, Block DA, et al. The 1987 revised ARA criteria for rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
19. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.
20. Masi AT, Rodnan GP, Medsger TA, et al. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1981;23:581–90.
21. Bohan A, Peter JB. Polymyositis and dermatomyositis. N Engl J Med 1975;292:344–7.
22. Mimori T, Ohosone Y, Hama N, et al. Isolation and characterization of cDNA encoding the 80-kDa subunit protein of the human autoantigen Ku (p70/p80) recognized by autoantibodies from patients with scleroderma-polymyositis overlap syndrome. Proc Natl Acad Sci USA 1990;87:1777–81.
23. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979;76:4350–4.
24. Lee JL, Ma H, Takano E, et al. Molecular diversity in amino-terminal domains of human calpastatin by exon skipping. J Biol Chem 1992;267:8437–42.
25. Harbets WJ, Winand WJ, Sillekens PTG, et al. Small nuclear RNA-associated proteins are immunologically related as revealed by mapping of autoimmune reactive B cell epitopes. Proc Natl Acad Sci USA 1989;86:4674–8.
26. Kohsaka H, Yamamoto K, Fujii H, et al. Fine epitope mapping of the human SS-B/La protein: identification of a distinct autoepitope homologous to a viral gag polyprotein. J Clin Invest 1990;85:1566–74.
27. Ohosone Y, Mimori T, Fujii T, et al. Autoantigenic epitopes of the B polypeptide of Sm small nuclear RNP particles. Identification of regions accessible only within the U1 small nuclear RNP. Arthritis Rheum 1992;35:960–6.
28. Reeves WH, Pierani A, Chou CH, et al. Epitopes of the p70 and p80 (Ku) lupus autoantigens. J Immunol 1991;146:2678–86.
29. Kuwana M, Kaburaki J, Mimori T, et al. Autoantigenic epitopes on DNA topoisomerase. I: Clinical and immunogenetic associations in systemic sclerosis. Arthritis Rheum 1993;36:1406–13.