EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 37, No. 6, 546-552, December 2005

Clinical significance of anti-filaggrin antibody recognizing uncitrullinated filaggrin in rheumatoid arthritis

Kyung-Ho Choi[1]*, Eun Bong Lee[2]*, Chang Dal Yoo[2], Han Joo Baek[2], Seong Wook Kang[2], Ki Chul Shin[2], Yun Jong Lee[2], Hyun Ah Kim[2], Ju-Hong Jeon[1], Chai-Wan Kim[1], Dong-Myung Shin[1], In-Gyu Kim[1,3] and Yeong Wook Song[2]

[1]Department of Biochemistry and Molecular Biology
Biology/Aging and Apoptosis Research Center (AARC)
[2]Department of Internal Medicine and Clinical Reasearch Institute
Seoul National University College of Medicine
Seoul 110-799, Korea
[3]Corresponding author: Tel, 82-2-740-8248;
Fax, 82-2-744-4534; E-mail, igkim@plaza.snu.ac.kr
*These authors contributed equally to this work.

Accepted 29 September 2005

Abbreviations: ADL, activity of daily living; AFA, anti-filaggrin antibody; AKA, anti-keratin antibody; ANA, anti-nuclear antibody; APF, anti-perinuclear factor; AS, ankylosing spondylitis; CRP, C-reactive protein; ESR, erythrocyte sedimentation ratio; OA, osteoarthritis; RA, rheumatoid arthritis; RF, rheumatoid factor; SLE, systemic lupus erythematosus

Abstract

Filaggrin is expressed in the cornified layer of epidermis and known to be one of the antigenic targets in rheumatoid arthritis. Although the citrulline residue in filaggrin is thought to be an antigenic determinant recognized by autoantibodies, the diagnostic sensitivity of synthetic citrullinated peptide is variable. To investigate the implication of anti-filaggrin antibodies recognizing uncitrullinated filaggrin in rheumatoid arthritis, we assayed antibody titers using unmodified recombinant filaggrin in the sera from 73 patients with rheumatoid arthritis, 150 patients with other connective tissue diseases and 70 normal controls. We also performed the correlation analysis between antibody titers and the clinical variables in patients with rheumatoid arthritis. Titers of IgG anti-filaggrin antibodies were significantly higher in rheumatoid arthritis patients compared to normal controls (*P* = 0.02), but not in patients with osteoarthritis, ankylosing spondylitis or systemic lupus erythematosus. IgG anti-filaggrin antibodies were more frequently found in patients with rheumatoid arthritis compared to normal controls (12.3% *vs* 1.4% respectively, *P* = 0.04). An anti-filaggrin antibody titer was correlated with visual analogue scale of pain, tender joint count, Ritchie articular index or C-reactive protein, but not with anti-nuclear antibody or rheumatoid factor. These results suggest that anti-filaggrin antibody recognizes the uncitrullinated filaggrin as an antigen and its titer correlates with clinical parameters, explaining the variable sensitivity of anti-filaggrin antibody test.

Keywords: autoimmune; cyclin citrullinated peptide; diseases; filaggrin; immunoenzyme techniques; rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease affecting around 1% of the general population (Kellgren, 1996). Although rheumatoid factor (RF) is helpful in diagnosis of RA (Arnett *et al.*, 1998), it can be found in patients with other rheumatic diseases or even healthy persons and is negative in 20-30% of RA patients (Schrohenloher *et al.*, 1997).

Various serologic markers were suggested to overcome the low specificity of the RF, including Sa, kalpastatin, antikeratin antibody (AKA) and antiperinuclear factor (APF) (Menard *et al.*, 1998). The AKA and APF, which are the antibodies against the cornified epithelium of rat esophagus and human buccal mucosa respectively, are known to be specific for RA (Nienhuis *et al.*, 1964; Young *et al.*, 1979). The antigen targets of both antibodies were suggested to be the same protein which is filaggrin in the human epidermis (Hoet *et al.*, 1991; Simon *et al.*, 1993; Sebbag *et al.*, 1995). Filaggrin is synthesized in the stratum granulosum as a large repeated (10-12 repeat of filaggrin monomers) and heavily phosphorylated precursor, profilaggrin, which is stored in the keratohyaline granules. After dephosphorylation and proteolysis, filaggrin monomer is released from profilaggrin and modified by peptidylarginine deiminase which converts the arginine resi-

dues found in filaggrin to the citrulline. Modified filaggrin is thought to be interacted and aggregated with the keratin intermediate filaments in keratinocytes, facilitating and guiding their alignment (Berthelot *et al.*, 1995).

Since the molecular identity of target antigen was elucidated, several approaches have been made to detect antifilaggrin antibody (AFA) using human filaggrin. Immunoblot tests using the filaggrin extract from human epidermis showed variable sensitivity ranging from 12.0-67.9%, with the specificity of 92.0-95.4% (Vincent *et al.*, 1998; Slack *et al.*, 1998). ELISA using filaggrin purified from human epidermis detected IgG AFA in 47% of RA patients (Palosuo *et al.*, 1998). Recently, ELISA using a cyclic citrullinated synthetic peptide derived from the sequence of human filaggrin showed a higher specificity than other methods (Schellekens *et al.*, 2000; Girelli *et al.*, 2004). However, the heterogeneity of extracted filaggrin or synthesized peptide sequence makes it difficult to get consistent results, and thus to evaluate the correlations between the presence of autoantibody and clinical data related to RA activity. In this study, we report that ELISA using unmodified recombinant filaggrin showed a 12.3% diagnostic sensitivity at a specificity of 95% which was correlated with visual analogue scale of pain, tender joint count, Ritchie articular index or C-reactive protein in RA patients.

Materials and Methods

Serum samples

Serum samples were obtained from 73 patients diagnosed as RA according to the revised criteria by American Rheumatism Association (Arnett *et al.*, 1998), 70 healthy controls and 150 patients with other connective tissue diseases including osteoarthritis (OA), ankylosing spondylitis (AS) and systemic lupus erythematosus (SLE) at Rheumatology Clinic, Seoul National University Hospital. Serum samples were stored at -70°C until assayed.

Expression and purification of the recombinant filaggrin monomer

Human filaggrin monomer cDNA was obtained by PCR amplification of the partial cDNA clone of profilaggrin (λHF11 provided by Dr. P. Steinert; Gan *et al.*, 1990). PCR was performed with sense primer (FilP) 5'-CATATGTTCCTCTACCAGGTGAGC-3' and antisense primer (FilN) 5'-TCTGGACATTCAGGATCTTAACTCGAG-3'. The amplified product was verified by sequencing (Sequenase kit; USB, Cleveland, OH) and subcloned into the corresponding sites of pET15b expression vector after digestion with *Nde* I and *Xho* I. The recombinant plasmid was introduced into the expression host, BL21 (DE3).

The recombinant human filaggrin was prepared by pET bacterial expression system (Novagen, Madison, WI). Induction and purification of the recombinant protein was performed according to manufacturer's instructions. Briefly, transformed cells were cultured in LB medium plus 100 g/ml ampicillin at 37°C. Filaggrin production was induced in the presence of 1 mM isopropyl β-D-thiogalactopyranoside (IPTG). The washed cells from 1-liter culture were resuspended in 10 ml of binding buffer (20 mM Tris-Cl, 0.5 M NaCl, 5 mM imidazole, pH 7.9) and lysed by sonication. The suspension was centrifuged at 39,000 × *g* for 20 minutes at 4°C. The supernatant was filtered through a 0.45 m membrane and loaded on 6 ml HisBind metal chelation resin (Novagen, Madison, WI) pre-charged with 50 mM NiSO4 and equilibrated with binding buffer. The column was washed with 60 ml binding buffer and then with 30 ml each of washing buffer containing 60 mM or 100 mM imidazole (20 mM Tris-Cl, 0.5 M NaCl, 60 mM or 100 mM imidazole, pH 7.9). The recombinant protein was eluted with 30 ml elution buffer (20 mM Tris-Cl, 0.5 M NaCl, 1 M imidazole, pH 7.9). The eluate was concentrated by ultrafiltration (Ultrfree-15: Millipore, Bedford, MA).

For the immunoblot analysis, protein samples were electrophoresed under denaturing condition, and transferred to a nitrocellulose membrane. The membrane was blocked with 5% skim milk in Tris-buffered saline (TBS) for an hour and then incubated with mouse antifilaggrin monoclonal antibody (Biomedical Technologies, Stoughton, MA) for an hour. Peroxidase-labelled anti-mouse Ig antibody (Dako, Glostrup, Denmark) was used as a secondary probe and visualization was performed with enhanced chemiluminescence (Amersham, Piscataway, NJ).

Immunoblotting with human sera

One microgram of recombinant filaggrin was run on SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. The membrane was cut into strips and blocked with 5% skim milk in TBS. The strips were incubated with the patient sera diluted 1:100 for an hour at room temperature. After washing several times, the strips were treated with peroxidase-labelled anti-human IgG antibody (Dako, Glostrup, Denmark) for an hour at room temperature. The color reaction was developed by 3,3-diaminobenzidine substrate (Sigma, St. Louis, MO).

ELISA

The recombinant filaggrin (1 g/well) was coated on

a 96-well immunoassay plate (Maxisorp; Nunc, Rockside, Denmark) at 4°C, overnight. After washing with phosphate buffered saline-0.05% Tween-20 (PBST), blocking was done with 1% bovine serum albumin in PBS at room temperature for 2 h. Then, the coated plate was incubated with 100 l/well of 1:100 diluted sera for 2 h. After washing 5 times, 100 l of alkaline phosphatase-conjugated goat anti-human IgG (1:4,000, American Qualex, La Mirada, CA) or IgM (1:4,000, Southern Biotechnology, Birmingham, AL) antibody was added to each well. After incubation for 2 h, the plate was washed 6 times and 100 l of 1 mg/ml ρ-nitrophenyl phosphate in dienthanolamine-$MgCl_2$ was added to each well. The reaction was stopped by adding 50 l of 3 M NaOH/well and optical density (OD) was read at a wavelength of 405 nm. OD measured on uncoated well was subtracted. The cut-off value was defined as 3 Standard Deviation (SD) above the mean of normal controls. Arbitrary units (a.u.) were obtained according to the formula; 1 a.u. = patient OD value/ cut-off value. All serum samples were assayed in duplicate.

Clinical evaluation in patients with RA

The following features were assessed in 73 patients with RA; functional class (Hochberg *et al.*, 1991), anatomical stage (Steinbocker *et al.*, 1949), duration of morning stiffness, grip strength (Lee *et al.*, 1974), pain (visual analogue scale, 10 cm), activity of daily living (ADL) (Callahan *et al.*, 1987), 68-joint count for tenderness, 66-joint count for swelling (Felson *et al.*, 1993), Ritchie articular index (Richie *et al.*, 1968), complete blood count, Westergren erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), titer of RF and antinuclear antibody (ANA). Joint X-ray survey was done for both shoulders, hands, knees, feet, cervical spine, lumbosacral spine and pelvis in all patients.

Statistical analysis

The data were analyzed using SAS statistical software (version 6.03). Student *t*-test was used for comparison of continuous variables. Chi-square test was applied for analysis of nominal variables. Correlation coefficients were measured between the titer of AFA and the clinical variables; Spearman correlation coefficients for functional class or anatomical stage and Pearson correlation coefficients for other variables. *P*-value less than 0.05 was considered significant.

Results

Purification of recombinant filaggrin

The protein sequences of monomeric filaggrin are shown in Figure 1. The recombinant filaggrin, tagged with histidine hexamer at the N-terminal end, was expressed mainly in the soluble form in host *E. coli* under the control of IPTG. The protein purified by Ni^{2+} column chromatography was pure enough to appear as a single band with 40 kD in size on SDS-PAGE, and the immunoblot revealed it to be a filaggrin (Figure 2).

Detection of AFA in human sera

For the detection and quantitation of AFA in patient sera, we designed an ELISA method using the purified recombinant filaggrin as a coating antigen. Sera from 70 normal controls, 73 RA patients and 150 patients with other connective tissue diseases (OA 50, AS 50, SLE 50) were tested at 1:100

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 F   L   Y   Q   V   S   T   H   E   Q   S   E   S   A   H   G   R   T   G   T   S   T   G   G   R   Q   G   S   H   H     30
TTC CTC TAC CAG GTG AGC ACT CAT GAA CAG TCT GAG TCC GCC CAT GGA CGG ACC GGG ACC AGC ACT GGA GGA AGA CAA GGA TCC CAC CAC
 Q   Q   A   R   D   S   S   R   H   S   T   S   Q   E   G   Q   D   T   I   H   G   H   R   G   S   S   S   G   G   R     60
AAG CAG GCA CGA GAC AGC TCC AGG CAC TCA ACG TCC CAA GAG GGT CAG GAC ACC ATT CAT GGA CAC CCG GGG TCA AGC AGT GGA GGA AGG
 Q   G   S   H   Y   E   Q   L   V   D   R   S   G   H   S   G   S   H   H   S   H   T   T   S   Q   G   R   S   D   A     90
CAG GGA TCC CAC TAC GAG CAA TTG GTA GAT AGA TCT GGA CAC TCA GGG TCT CAT CAC AGC CAC ACC ACA TCC CAG GGA AGG TCT GAT GCC
 S   H   G   H   S   G   S   R   S   A   S   R   Q   T   R   N   D   E   Q   S   G   D   G   S   R   H   S   G   S   R    120
TCC CAT GGG CAC TCA GGA TCC AGA AGT GCA AGC AGA CAA ACT CGT AAC GAT GAA CAA TCA GGA GAC GGC TCC AGG CAC TCA GGG TCG CGT
 H   H   E   A   S   S   R   A   D   S   S   G   H   S   Q   V   G   Q   G   Q   S   E   G   P   R   T   S   R   N   W    150
CAC CAT GAA GCT TCC TCT CGG GCC GAC AGC TCT GGA CAC TCG CAG GTG GGC CAG GGA CAA TCA GAG GGG CCC AGG ACA AGC AGG AAC TGG
 G   S   S   F   S   Q   D   S   D   S   Q   G   H   S   E   D   S   E   R   W   S   G   S   A   S   R   N   H   H   G    180
GGA TCC AGT TTT AGC CAG GAC AGT GAC AGT CAG GGA CAC TCA GAA GAC TCT GAG AGG TGG TCT GGG TCT GCT TCC AGA AAC CAT CAT GGA
 S   A   Q   E   Q   L   R   D   G   S   R   H   P   R   S   H   Q   E   D   R   A   G   H   G   H   S   A   D   S   S    210
TCT GCT CAG GAG CAG CTA AGA GAT GGC TCC AGA CAC CCC AGG TCC CAT CAA GAA GAC AGA GCT GGT CAT GGG CAC TCT GCA GAC AGC TCC
 R   Q   S   G   T   R   H   T   Q   T   S   S   G   G   Q   A   A   S   S   H   E   Q   A   R   S   S   A   G   D   R    240
AGA CAA TCA GGC ACT CGT CAC ACA CAG ACT TCC TCT GGT GGA CAG GCT GCA TCA TCC CAT GAA CAG GCA AGA TCA AGT GCA GGA GAA AGA
 H   G   S   H   H   Q   Q   S   A   D   S   S   R   H   S   G   I   G   H   G   Q   A   S   S   A   V   R   D   S   G    270
CAT GGA TCC CAC CAC CAG CAG TCA GCA GAC AGC TCC AGA CAC TCA GGC ATT GGG CAC GGA CAA GCT TCA TCT GCA GTC AGA GAC AGT GGA
 H   R   G   Y   S   G   S   Q   A   S   D   N   E   G   H   S   E   D   S   D   T   Q   S   V   S   A   H   G   Q   A    300
CAC CGA GGG TAC AGT GGT AGT CAG GCC AGT GAC AAT GAG GGA CAT TCA GAA GAC TCA GAC ACA CAG TCA GTG TCA GCC CAC GGA CAG GCT
 G   S   H   Q   Q   S   H   Q   E   S   A   R   G   R   S   G   E   T   S   G   H   S   G   S                            324
GGG TCC CAT CAG CAG AGC CAC CAA GAG TCC GCA CGT GGC CGG TCA GGG GAA ACG TCT GGA CAT TCA GGA TCT
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Figure 1. The amino acids sequence of human recombinant filaggrin used in this study.

dilution. As both APF and AKA were mainly IgG isotype, IgG AFA was tested first. The average AFA titer of RA patients was significantly higher than that of normal controls (mean ± SEM; 0.417 ± 0.100 a.u. versus 0.179 ± 0.028 a.u., P = 0.02), whereas other disease groups showed the similar AFA titer (Table 1, Figure 3A). In contrast, IgM AFA titer showed no difference between RA patients and normal controls (0.279 ± 0.029 a.u. versus 0.209 ± 0.026 a.u., P = 0.08) (Figure 3B). When the cut-off value was defined as 3 SD above the mean of normal controls, 9 from 73 RA sera (12.3%) were positive for IgG AFA, whereas 1 from 70 normal sera (1.4%), 1 from 50 OA sera (2%), 2 from 50 AS sera (4%), and 4 from 50 SLE sera (8%) were positive. The sensitivity and specificity of the IgG AFA test in RA patients were calculated as 12.3%, 96.0% respectively. Immunoblot with RA sera showed that AFA in human sera binds to the recombinant filaggrin (Figure 4).

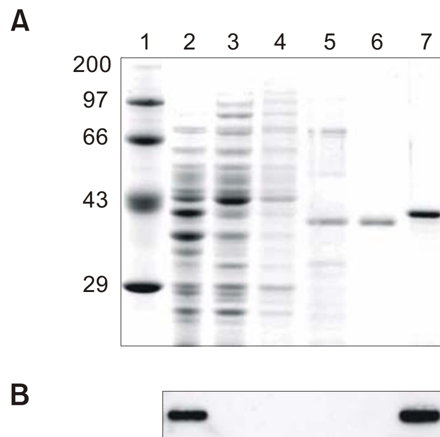

Figure 2. Purification of the recombinant filaggrin. The lysed cell extract was loaded on Ni^{2+} column. During the washing, imidazole concentration increased up to 100 mM in a step gradient manner. The 40 kD target protein was eluted at 1M imidazole and electrophoresed on a 8% SDS-polyacrylamide gel. The fractionated bands were stained with Coomassie blue (A) or analyzed by immunoblot with mouse anti-human filaggrin antibody (B). 1, molecular weight marker (kD); 2, the lysed cell extract; 3, the flow-through; 4, 5 mM imidazole; 5, 60 mM imidazole; 6, 100 mM imidazole; 7, 1 M imidazole.

AFA titer and clinical features of RA patients

To evaluate the clinical significance of AFA positivity and titer, we categorized the clinical features of RA patients and examined the correlation with AFA titer. The baseline clinical characteristics of 73 RA patients were as Table 2. At the study entry, 46 patients were receiving at least one of the disease modifying anti-rheumatic drugs (hydroxychloroquine 34, methotrexate 30, azathioprine 6, sulfasalazine 5, bucil-

Table 1. IgG AFA titer in RA and other connective tissue diseases. The IgG AFA titer of RA patients was significantly higher than that of normal controls.

Disease	IgG AFA (a.u., mean ± SEM)	P-value
Rheumatoid arthritis (n = 73)	0.417 ± 0.100	0.03
Osteoarthritis (n = 50)	0.189 ± 0.028	0.80
Ankylosing spondylitis (n = 50)	0.250 ± 0.043	0.15
Systemic lupus erythematosus (n = 50)	0.266 ± 0.053	0.12
Normal controls (n = 70)	0.179 ± 0.028	

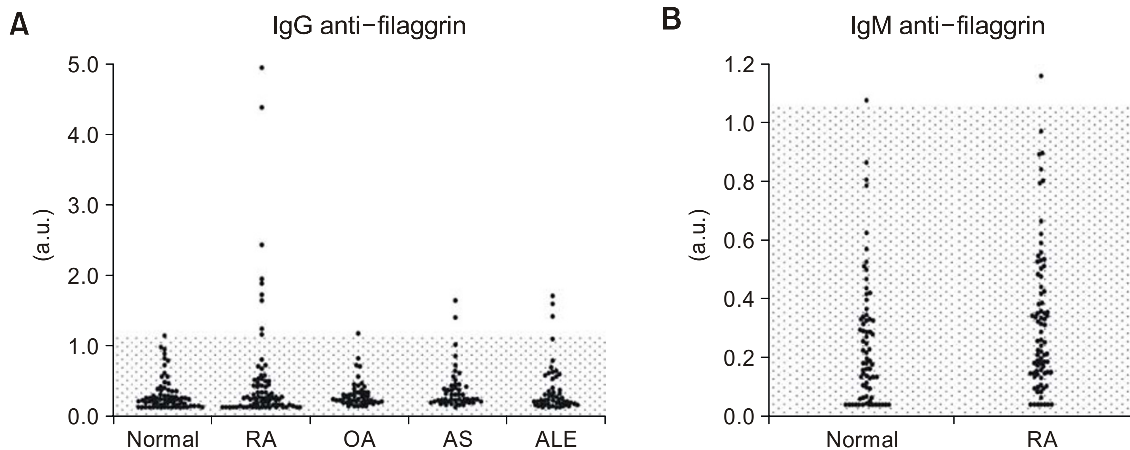

Figure 3. IgG AFA in connective tissue diseases (A) and IgM AFA (B) in RA. Shaded area represents the range of optical density value lower than the cut-off value of 1 arbitrary unit (a.u.). The cut-off value was chosen as 3 SD above the mean of normal control optical density values. IgG antifilaggrin titer was significantly higher in RA patients compared with that of noraml controls (P = 0.02). There was no significant difference of IgM AFA titer between RA patients and normal controls.

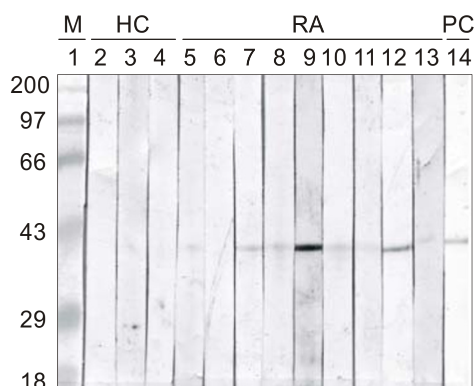

Figure 4. Reactivity of RA sera to the recombinant filaggrin in immunoblot. After the electrophoretic separation and transfer of 1 g recombinant filaggrin, 3 healthy control sera (lane 2-4), 9 RA sera (lane 5-13) were tested at 1:100 dilution. Lane 14 was reacted with mouse anti-human filaggrin monoclonal antibody as a positive control. M, marker; HC, healthy control; RA, rheumatoid arthritis; PC, positive control.

Table 2. Baseline clinical chracteristics of patients with RA.

Clinical charateristics	*n* = 73
Female (%)	61 (83.6%)
Age (years, mean ± SD)	49.6 ± 12.6
Duration (months, mean ± SD)	113.3 ± 114.1
Rheumatoid factor (%)	52 (71%)
DMARDs (%)*	46 (63%)

*Disease modifiying anti-rheumatic drugs.

lamine 2). Low dose prednisolone was administered in 27 patients.

IgG AFA was positive in 9 (12.3%) out of 73 RA patients. With respect to the RF, eight patients showed positive AFA (15.4%) in 52 RF positive patients, compared with only one (4.8%) in 21 RF negative patients (P = 0.09). All patients with positive AFA were suffering from RA longer than 12 months. AFA was positive in 7 (13.5%) out of 52 patients with one or more marginal erosions in joint X-rays, while it was positive in 2 (10%) out of 20 patients without erosion (P = 0.43).

When correlation analysis was performed between AFA titers and clinical variables in AFA positive RA patients, the severity of pain measured by visual analogue scale (r = 0.73, P = 0.03), tender joint count (r = 0.76, P = 0.02), Ritchie articular index (r = 0.89, P = 0.001), CRP (r = 0.82, P = 0.01) and platelet count (r = -0.75, P = 0.03) showed significant correlation (Table 3).

Table 3. Correlation between AFA titer and clinical variables in AFA positive RA patients.

Variables	r	*P*
Functional class	0.00	1.00
Anatomical stage	0.25	0.52
Morning stiffness	−0.19	0.62
Grip Strength (right)	−0.27	0.48
Grip Strength (left)	−0.37	0.32
Pain (VAS)	0.73	0.03
ADL	0.56	0.12
Tender joint count	0.76	0.02
Swollen joint count	0.31	0.42
Ritchie index	0.89	0.001
C-reactive protein	0.82	0.01
White blood cell	0.43	0.28
Platelet	−0.75	0.03
Westergren ESR	0.52	0.18
RF titer	0.08	0.84

ADL, activity of daily living; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; VAS, visual analogue scale

Discussion

AFA is the autoantibody to filaggrin in human epidermis and detected in the RA patients by immunoblot analysis and ELISA using human epidermal extracts or synthetic citrullinated peptide as an antigen (Palosuo *et al.*, 1998; Vincent *et al.*, 1998; Schellekens *et al.*, 2000). Although the specificity of the tests was high (92.0-95.4%), the sensitivity of the tests was variable (12.0-67.9%) probably due to the difference in source of filaggrin extracted or in sequences of citrullinated peptide from various providers.

Human filaggrin consists of a heterogeneous population of monomers of different sequences and charges. Furthermore, human filaggrin shows considerable polymorphic sequence variations between individuals (McKinley-Grant *et al.*, 1989; Gan *et al.*, 1990). In addition, the post-translationally modified arginine residue in filaggrin monomer is thought to be an antigenic determinant recognized by AFA (Schellekens *et al.*, 1998). However, the number and location of modified arginine in filaggrin repeats have not been determined. Our study was conducted to test whether AFA could react with unmodified human filaggrin and whether the presence of this antibody in RA patients correlates with the clinical and laboratory profiles.

To guarantee the antigenic homogeneity of recombinant filaggrin which is not modified, we used bac-

terial expression system. Because proteolytic cleavage site of filaggrin precursor protein has not been exactly determined yet, we designed the filaggrin monomer for expression as suggested previously (Gan *et al.*, 1990). The filaggrin monomer includes the linker and proposed filaggrin sequences. With our ELISA system, the sensitivity of AFA in RA patients was 12.3% with the specificity of 95%. High specificity of the test reconfirmed the previous reports (Vincent *et al.*, 2005) and suggested that the AFA test could be used to differentiate the RA from other inflammatory arthritis. The low positivity rate can be explained as follows. First, there is heterogeneity of the filaggrin monomers which show the sequence variability in 30-40% of amino acids (Gan *et al.*, 1990). Second, recombinant filaggrin might not have the appropriate tertiary structure which is needed for the antibody binding. Third, citrullination may explain two third of the AFA-positive sera (Schellenkens *et al.*, 1998; Kroot *et al.*, 2000). Fourth, the prevalence of the AFA in Korean patient group may be low compared with those of other ethnic groups. The reported prevalence of AFA was variable (12.0-67.9%) and in case of AKA, the Greek patients with RA had low positivity (16%) in spite of consistently high positivity (36-69%) in other ethnic groups.

AKA and APF could be positive at subclinical stage of RA (Paimela *et al.*,1992) and IgG AKA was found in 38% of RA patients whose disease duration is less than one year (Aho *et al.*, 1993). AKA and APF were suggested to be early diagnostic markers of RA. However, unlike AKA or APF, we could not detect AFA among 8 patients in whom the duration of the disease was less than 1 yr. AKA and APF were reported to show higher positivity rate in patients with destructive RA (Westgeest *et al.*, 1987; Kurki *et al.*, 1997). However, there was no difference in AFA positivity between the patients with and without marginal erosion in our patient group. It was reported that the frequency of RF is increased in AKA or APF positive patients (Paimela *et al.*, 1992). In this study, the frequency of RF positivity tended to be higher in AFA positive patients compared with AFA negative patients (89% versus 67%, $P = 0.18$).

The association of AFA with the clinical and laboratory profiles in RA patients has not been well described. Recently, only one study reports a correlation between the presence of anti-citrullinated peptide antibodies and the functional disability (Bas *et al.*, 2003). We found positive correlation between AFA titer and several clinical features including pain in visual analogue scale, tender joint count, Ritchie articular index and CRP. Platelet count was negatively correlated with AFA titer. The clinical significance of negative correlation between platelet count and AFA titer remains to be explained.

In conclusion, we showed that AFA recognizes the recombinant filaggrin monomer which is not citrullinated as an antigen, explaining the variable sensitivity of AFA test. Antibody titer was significantly increased in RA patients and showed correlation with pain in visual analogue scale, tender joints count, Ritchie articular index and CRP.

Acknowledgement

This work was supported by Seoul National University Hospital (03-1997-010-1), Ministry of Health and Welfare (HMP-97-B-2-0011) and Korea Science and Engineering Foundation (KOSEF; R11-2002-097-07003-0 and R01-2005-000-10364-0) through the Center for Aging and Apoptosis Research. J.H.J., C.W.K. and D.M.S. were supported by the graduate program of BK21 project, administrated by the Ministry of Education and Human Resources Development.

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