Autoantibody against ribosomal protein L14 in patients with systemic lupus erythematosus

H. Hasegawa[1], T. Uchiumi[2], T. Sato[3], A. Saito[1], M. Nakano[1], F. Gejyo[1]

[1]Department of Medicine (II), Niigata University School of Medicine, Niigata; [2]Institute of High Polymer Research, Faculty of Textile Science and Technology, Shinshu University, Ueda; [3]Sato Medical Clinic, Joetsu, Japan.

Abstract

Objective

To isolate a specific antibody against ribosomal protein L14 and to assess the relationship of this antibody with some of the clinical features in patients with systemic lupus erythematosus (SLE).

Methods

We screened the sera of SLE patients by immunoblotting analysis using rat total ribosomal proteins as antigen to determine whether sera had antibody activity against ribosomal proteins other than the P, S10, and L12 proteins. The sera from 2 patients had antibody activity against a 30-kDa ribosomal protein. This antigenic protein was identified to be ribosomal protein L14 by two-dimensional gel electrophoresis and immunoblotting, so the antibody against L14 was tested by immunoblotting analysis using glutathione-S-transferase fusion human-L14 protein (GST-L14) as the antigen. We examined sera from 126 patients with SLE, and as controls sera from 67 patients with dermatomyositis and polymyositis (DM/PM), 71 patients with systemic sclerosis (SSc), and 74 healthy donors.

Results

Antibody activity against GST-L14 was detected in 7 out of 126 SLE, but not in any of the DM/PM, PSS, or healthy controls.

Conclusion

Antibody against ribosomal protein L14 was specifically detected in sera from patients with SLE. Although this antibody activity was not so prevalent in the patients with SLE, it might be one of the useful tools for diagnosis of SLE.

Key words

Autoantibody, ribosomal protein L14, and systemic lupus erythematosus.

Hisashi Hasegawa, MD; Toshio Uchiumi, PhD; Takehiro Sato, MD; Akihiko Saito, MD; Masaaki Nakano, MD; and Fumitake Gejyo, MD.

Please address reprint requests and correspondence to: Hisashi Hasegawa, MD, Department of Molecular Sciences, University of Tennessee Health Science Center, 858 Madison Avenue, Suite G01, Memphis, TN 38163, USA.
E-mail: hhasegawa@utmem.edu

Received on May 24, 2001; accepted in revised form on October 8, 2001.

Introduction

Systemic lupus erythematosus (SLE) is characterized by the production of diverse autoantibodies against nuclear and cytoplasmic components (1, 2). Antibodies against ribosomal P, L12 and S10 proteins are detected at various levels specifically in patients with SLE (3-6). Anti-ribosomal P antibodies have been reported to be related to a specific clinical manifestation, central nervous system involvement (CNS lupus) (7), although this association is still contraversial. We have investigated antibody activity to ribosomal proteins in lupus sera and have been aware of the presence of autoantibodies against ribosomal proteins other than P, L12 and S10 proteins in some lupus sera. In this study we identified one of these autoantibodies and demonstrated that it recognizes L14 ribosomal protein.

Materials and methods

Screening of antibodies against ribosomal proteins

Lupus sera were screened for antibody activity against rat ribosomal proteins other than the P, S10, and L12 proteins by immunoblotting. Two SLE patient (patient nos. 6 and 7) sera contained antibody to a 30-kDa ribosomal protein. This antigenic ribosomal protein was identified by two-dimensional gel electrophoresis and immunoblotting.

Patients

Sera were obtained from 126 Japanese patients (age range 15-58 years) with systemic lupus erythematosus as defined by the American College of Rheumatology criteria (8). Sera from 67 patients (age range 15-71 years) with polymyositis/dermatomyositis (PM/DM) as defined by the criteria of Bohan *et al*. (9), from 71 patients (age range 16-65 years) with systemic sclerosis (SSc) defined by the American College of Rheumatology criteria (10), and from 74 healthy donors (age range 18-59 years) were used as disease and normal controls. All patients had been treated in the Niigata University Hospital and serum samples were taken at the time of admission and stored at -80°C until use.

Ribosomal proteins

The total ribosomal proteins (TP80) were prepared from rat livers by homogenization and discontinuous gradient ultracentrifugation as described by Ogata & Terao (11).

Electrophoresis and immunoblotting

One-dimensional polyacrylamide-SDS gel electrophoresis (12) and two-dimensional gel electrophoresis (13) were performed as previously described. Immunoblotting was carried out as described previously (14, 6) using patients'sera diluted 1:100 as the primary antibody, followed by horseradish peroxidase-conjugated anti-human IgG diluted 1:800 as the secondary antibody (DAKO, Gloostrup, Denmark). The antibody-bound position was visualized using a standard substrate solution.

Plasmid construction and recombinant human L14 protein

A full-length complementary DNA (cDNA) clone for human ribosomal protein L14 (15) was a kind gift of Dr. M. Tanaka (The National Institute of Bioscience and Human-Technology, Ibaraki, Japan). The region encoding the whole L14 molecule was amplified by polymerase chain reaction (PCR) and inserted between EcoRI and XhoI sites located downstream of the GST sequence in the pGEX-KG expression vector (16), which was kindly provided from Dr. Robert A. Orlando (University of California,San Diego,CA). The glutathione S-transferase (GST) fusion protein was expressed in *Escherichia coli* (DH5) and purified using Glutathione Sepharose 4B (Amersham Pharmacia Biotech AB, Uppsala, Sweden) as described (17).

Clinical and serological parameters in the patients with antibody against L14

Clinical and laboratory findings at the time of serum sampling were collected retrospectively. Serum levels of immunogloblins (IgG, IgA, IgM) and complements (C3, C4, CH50) were obtained using standard hospital techniques. Anti-ds DNA antibody was examined by an indirect immunofluo-

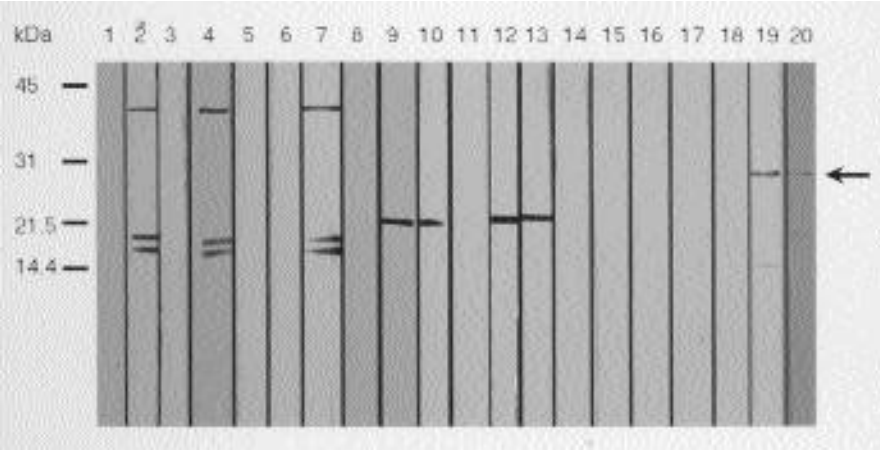

Fig. 1. Screening of antibodies against ribosomal proteins in lupus sera. Total ribosomal proteins were electrophoresed on 16% polyacrylamide-SDS slab gels and transferred to nitrocellulose membranes. Nitrocellulose membranes were incubated with 1/100 diluted lupus sera. Sera in lanes 2,4,and 7 contained anti-ribosomal P antibody activity, and sera in lanes 9,10,12,and 13 contained antibody activity against a 20-kDa ribosomal protein that seemed to be either S10 or L12. Sera from patient no. 7 (lane 19) and no. 6 (lane 20) contained antibodies against a 30-kDa protein (arrow).

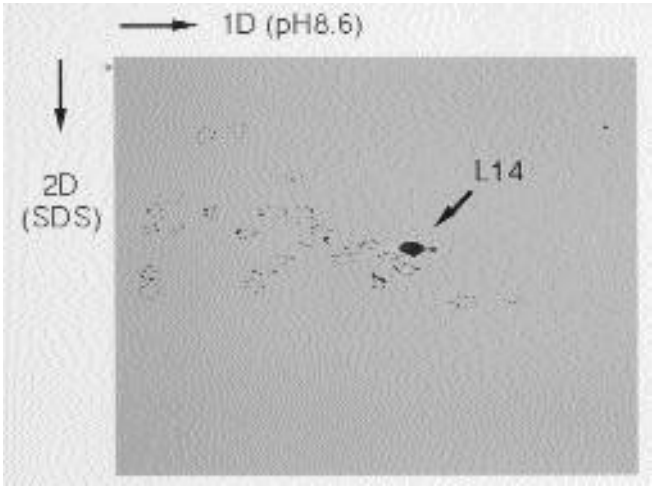

Fig. 2. Reactivity of serum from an SLE patient (no. 7) with L14 ribosomal protein separated by two-dimensional gel electrophoresis. Total ribosomal proteins were separated by two-dimensional polyacrylamide gel electrophoresis system with basic urea gel in the first dimension (1D) (pH 8.6) and sodium dodecyl sulfate (SDS) gel in the second dimension. The separated proteins were transferred from the gel to a nitrocellulose membrane, followed by immunoblotting with patient serum. The position corresponding to reactivity with L14 ribosomal protein is indicated by the arrow.

rescence test on the *Crithidia luciliae*, radioimmunoassay using the Farr, or enzyme-linked immunosorbent assay (ELISA). Anti-Sm, anti-RNP, anti-SS-A (Ro), anti-SS-B (La), and anti-Scl-70 (Topo-I) antibodies were examined by the double immunodiffusion method or ELISA. Anti-histone H1 antibody was measured by immunoblotting analysis using purified histone H1 derived from calf thymus (Roche Molecular Biochemicals, Mannheim, Germany) as the antigen according to the method described by Schett *et al.* (18).

Statistical analysis

The difference between disease groups in the prevalence of antibody against ribosomal protein L14 was evaluated by Fisher's exact probability test. A level of $P < 0.05$ was accepted as statistically significant.

Results

Screening of antibodies to rat ribosomal proteins

We screened sera from patients with SLE to detect antibodies against ribosomal proteins. Immunoblotting analysis was performed using rat total ribosomal proteins as antigens. The sera from two SLE patients (patient nos. 6 and 7) contained antibodies to a 30-kDa ribosomal protein (Fig. 1). To identify this 30-kDa ribosomal protein, we analyzed total protein from rat liver ribosomes by two-dimensional gel electrophoresis, followed by immunoblotting with serum from SLE patient (no. 7) serum. As shown in Figure 2, this serum reacted with a specific ribosomal protein. This protein was identified as the large subunit protein L14, according to the numbering system of mammalian ribosomal proteins on two-dimensional gel (13). Although the molecular weight of rat L14 is estimated to be 23 kDa from its amino acid sequence (19), the molecular weight estimated from mobility of this protein on polyacrylamide-SDS gel was 30 kDa.

Reactivity of antibodies to human L14 ribosomal protein

GST-L14 fusion protein (GST-L14) was expressed, purified, and resolved by 16 % polyacrylamide-SDS gel electrophoresis. As shown in Figure 3, a 52-kDa protein corresponding to the predicted size of GST-L14 was detected. Since it was recognized by both rabbit polyclonal IgG anti-GST antibody (Upstate Biotechnology, Lake Placid, NY) and SLE patient serum (no. 7), this 52-kDa protein was confirmed to be GST-L14. Among serum samples from 126 SLE patients, 7 had reactivity against GST-L14 by immunoblotting (Fig. 4).

To eliminate the influence of anti-ds DNA antibody against DNA that may bind to ribosomal protein L14, we performed immunoblotting using these 7 sera pre-incubated with 50 units/ml of DNase I (Nippon gene, Tokyo, Japan) for 1 hour at 37°C. Antibody activity against GST-L14 was still present in these 7 sera after DNA digestion (not shown). This suggests that the antibody activity against L14 is not via L14-binding DNA and anti-ds DNA antibody.

In contrast, no GST-L14 reactivity was detected in sera from 67 patients with PM/DM, 71 patients with SSc, or 74 healthy donors by immunoblotting.

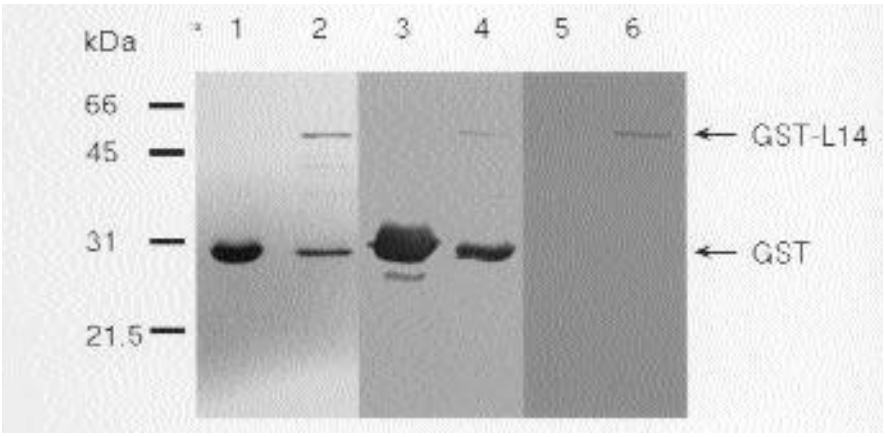

Fig. 3. Expression and identification of GST and GST-L14. Samples containing purified GST (lanes 1, 3, and 5) and GST-L14 (lanes 2, 4, and 6) were separated on SDS-PAGE. The gel was stained with Coomassie Brilliant Blue (lanes 1 and 2). The separated GST and GST-L14 were transferred from gel to nitrocellulose, followed by immunoblotting with anti-GST antibody (lanes 3 and 4) as the primary antibody and anti-rabbit immunoglobulins (DAKO, Glostrup, Denmark) as the second antibody, and with serum from patient no. 7 (lanes 5 and 6). In lanes 4 and 6,a 52-kDa protein was reactive with both anti-GST antibody and patient serum.

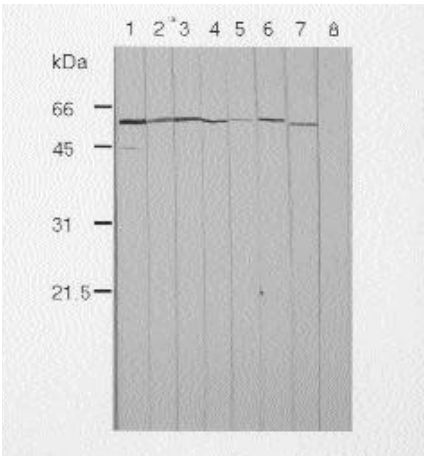

Fig. 4. Immunoblotting of GST-L14 with lupus patients sera. GST-L14 was resolved by 16% polyacrylamide-SDS slab gel electrophoresis and transferred to nitrocellulose membranes. Nitrocellulose membranes were incubated with 1/100 diluted sera from 126 patients with SLE, 67 patients with PM/DM, 71 patients with SSc, and 74 health donors. 7 lupus sera contained antibody activity against GST-L14 (lanes 1 to 7). Serum from a healthy donor (lane 8) did not react with GST-L14.

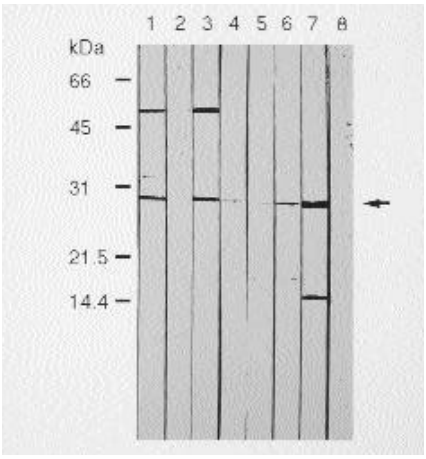

Fig. 5. Immunoblotting of total ribosomal proteins (TP80) from rat liver with lupus patients' sera. TP80 was resolved by 16% polyacrylamide-SDS slab gel electrophoresis and transferred to nitrocellulose membranes. Nitrocellulose membranes were incubated with 7 lupus sera samples containing antibodies against GST-L14 (lanes 1 to 7) and serum from a healthy donor (lane 8). Arrowhead indicates the molecular weight of L14.

Therefore, anti-GST-L14 antibody was specifically found in the SLE patients ($P < 0.05$). Although 6 of these 7 sera samples contained reactivity to a 30-kDa ribosomal protein that was assumed to be rat ribosomal protein L14, one serum sample (patient no. 2) did not react with rat ribosomal protein L14 by immunoblotting using rat total ribosomal proteins as the antigen (Fig. 5).

Clinical features of patients with anti-GST-L14 antibody

Table I shows clinical features of the SLE patients with anti GST-L14 antibody. Although these 7 patients did not have any common clinical features, skin, joint, and hematological involvement were frequently observed. Six out of 7 patients had low complement levels, and all 7 had anti-ds DNA antibody. Anti-histone H-1 antibodies were detected in only 2 sera out of 7 (Table II).

Discussion

Ribosomal components including P proteins, S10 protein, L12 protein, and 28S rRNA (20) are known to be autoantigens in lupus sera, although the majority of autoantigens in SLE are components of the nucleus such as DNA, small nuclear ribonucleoproteins, and histones. We have demonstrated that a portion of anti-Sm antibodies cross-react with the ribosomal S10 protein (21,22). Tsuzaka *et al.* previously reported that some anti-ds DNA antibodies cross-react with the ribosomal S1 protein (23). Therefore, antibody activity against some ribosomal proteins might result from cross-reactivity against nuclear components. In this study, we found a novel autoantigen that we identified to be the ribosomal protein L14, and demonstrated that autoantibody against this protein was detected specifically in lupus sera. At first we predicted that this antibody activity was due to cross-reactivity of the antibody against some nuclear components. Comparison of the amino acid sequence of human ribosomal L14 with the BLAST database indicated that L14 had amino acid sequence similarity to histone H1 (15, 24, 25). However, in this study only 2 out of 7 sera had anti-histone H1 antibody activity. It is not likely that all of the antibody activity against ribosomal L14 was due to anti-histone H1 antibody cross-reactivity. However, histone proteins are one of the main targets of lupus autoantibodies, and are also basic proteins like ribosomal L14 (26). Antibodies against other histone proteins might also thus be expected to cross-react with ribosomal L14.

We used GST-L14 fusion protein as the antigen to detect antibody against human L14 protein. As shown in Figure 3, GST-L14 protein solution contained certain amount of GST, too. However, none of the 7 sera that were reactive with GST-L14 reacted with GST protein alone (Fig. 4). This finding strongly suggests that the antibody activity against human L14 protein is specific and GST is not antigenic.

Table I. Clinical charcteristics at the time of serum sampling of SLE patients with anti-GST-L14 antibodies.

Patient No.	Age/Sex	Involved organs
1.	28/F	CNS (menengitis, transverse myelitis), skin, joints
2.	48/F	kidney, hematological, joints
3.	26/F	kidney, hematological, skin, heart (mitral regurgitation)
4.	31/F	kidney, hematological
5.	45/F	skin, joints
6.	34/F	hematological, pleuritis, skin, joints, liver
7.	26/F	hematological, skin, joints, liver

Skin involvement includes oral ulcers, rash, and discoid rash,
Hematological disorders includes hemolytic anemia, leukopenia, lomphopenia and thrombopenia.
Kidney involvement includes proteinuria, more than 0.5 g/day or cellular casts.
Liver involvement includes elevation of serum level of glutamic oxalacetic transaminase (GOT) or glutamic pyruvic transaminase (GPT) more than two time the normal range each.

Interestingly, all 7 patients who were positive for anti-GST-L14 antibody had anti-ds DNA antibodies. The effect of anti-ds DNA antibody to ribosomal L14 should be considered in two possibilities. The first is that anti-ds DNA antibodies can react with the DNA bound to basic proteins such as histone proteins. Ribosomal L14 is also a basic protein and may bind to DNA in sera. However, in the present study DNase-treated sera retained antibody activity against GST-L14 equivalent to non-DNase-treated sera. Therefore, it is unlikely that anti-ds DNA antibody reacts with L14 via L14-binding DNA. Anti-ds DNA antibody is often detected in the sera from patients with SLE, and some anti-ds DNA antibody has been reported to cross-react with ribosomal protein S1 (23). The other possibility is that some anti-ds DNA antibodies might cross-react with ribosomal protein L14. Although this hypothesis is interesting and fascinating, the direct cross-reaction of anti-ds DNA antibody against L14 has not been confirmed in this study and should be elucidated.

Ribosomal protein L14 is a basic protein, and its molecular weight is 23.6 kDa in humans. A significant identity for human ribosomal protein L14 is observed with rat ribosomal protein L14 (85% identity), with the exception of the C-terminal region. The cDNA of human L14 has a trinucleotide-repeat (GCT; alanine) region at nucleotides 465 to 509 (15), and there are some allelic polymorphisms in the trinucleotide repeat length (27). The main epitopes of L14 may be located in the N-terminal region of this protein in the majority cases, although the serum of one SLE (no. 2) reacted only with GST-L14, and not with rat L14: no. 2 serum might detect a neoepitope that was yielded by the fusion of GST and human L14 ribosomal proteins. Further epitope analyses of L14 and cross-reaction studies are necessary to better characterize this antibody.

Although anti-P antibodies have been reported to be associated with central nervous system involvement, no special clinical features were found to be associated with antibodies against other ribosomal proteins. Anti-L14 antibody may not be related to any specific clinical features; however, in our patients' group with anti-L14 antibody, clinical findings such as skin, joint, and hematological involvement, hypocomplementemia, and anti-ds DNA antibody were frequently observed. In studies on the Japanese population, these clinical findings were observed in active disease at a similar rate (28, 29) to those in the patients' group with anti-GST-L14 antibody in this study. The appearance of anti-L14 antibody may reflect the disease activity in SLE patients.

In summary, some ribosomal proteins are autoantigenic in lupus sera and ribosomal protein L14 was found to be one such autoantigen in this study. Although the prevalence of anti-L14 antibody is low, it is detected specifically in patients with SLE and may represent another useful diagnostic marker of SLE.

Acknowledgments

We are grateful to Dr. Manami Tanaka (The National Institute of Bioscience and Human-Technology, Ibaraki, Japan) for providing the cDNA clone for human L14 ribosomal protein. We also thank Dr. Robert A. Orlando (Division of Cellular and Molecular Medicine, University of California, San Diego,

Table II. Immunological data at the time of serum sampling of SLE patients with ant-GST-L14 antibodies.

(normal range) Patient no.	IgG (mg/dl) (870-1700)	IgA (mg/dl) (110-410)	IgM (mg/dl) (35-220)	C3 (mg/dl) (55.3-140)	C4 mg/dl) (13.2-41.7)	CH50 (U/dl) (28-53)	Coexistent autoantibodies
1.	1977	318	76	41	10	24	anti-dsDNA
2.	2696	339	1150	15	11	14	anti-dsDNA, anti-histone H1
3.	2490	334	86	28	7	13	anti-dsDNA, anti-Sm, anti RNP, anti-histone H1
4.	1055	299	28	37	21	25	anti-dsDNA
5.	2470	585	142	84	24	53	anti-dsDNA
6.	2587	508	133	24	12	15	anti-dsDNA, anti-SS-A
7.	3576	358	145	40	11	29	anti-dsDNA, anti-SS-A

CA) for the gift of the pGEX-KG vector, and Wallace S. McCloy III for reviewing the manuscript.

References

1. CHRISTIAN CL, ELKON KB: Autoantibodies to intracellular proteins. Clinical and biologic significance. *Am J Med* 1986; 80: 53-61.
2. TAN EM: Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv Immunol* 1989; 44: 93-151.
3. ELKON KB, PARNASSA AP, FOSTER CL: Lupus autoantibodies target ribosomal P proteins. *J Exp Med* 1985; 162: 459-71.
4. BONFA E, PARNASSA AP, RHOADS DD, ROUFA DJ, WOOL IG, ELKON KB: Antiribosomal S10 antibodies in humans and MRL/lpr mice with systemic lupus erythematosus. *Arthritis Rheum* 1989; 32: 1252-61.
5. SATO T, UCHIUMI T, OZAWA T, *et al.*: Autoantibodies against ribosomal proteins found with high frequency in patients with systemic lupus erythematosus with active disease. *J Rheumatol* 1991; 18: 1681-4.
6. SATO T, UCHIUMI T, KOMINAMI R,ARAKAWA M: Autoantibodies specific for the 20-KDal ribosomal large subunit protein L12. *Biochem Biophys Res Commun* 1990; 172: 496-502.
7. BONFA E, GOLOMBEK SJ, KAUFMAN LD, *et al.*: Association between lupus psychosis and anti-ribosomal P protein antibodies. *N Engl J Med* 1987; 317: 265-71.
8. 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.
9. BOHAN A, PETER JB: Polymyositis and dermatomyositis (first of two parts). *N Engl J Med* 1975; 292: 344-7.
10. PRELIMINARY CRITERIA FORTHE CLASSIFICATION OF SYSTEMIC SCLEROSIS (SCLERODERMA): Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum* 1980; 23: 581-90.
11. OGATA K, TERAO K: Analytical methods for ribosomal proteins of rat liver 40 S and 60 S subunits by "three-dimensional" acrylamide gel electrophoresis. *Methods Enzymol* 1979; 59: 502-15.
12. LAEMMLI UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227: 680-5.
13. UCHIUMI T, KIKUCHI M, TERAO K, IWASAKI K, OGATA K: Cross-linking of elongation factor 2 to rat-liver ribosomal proteins by 2-iminothiolane. *Eur J Biochem* 1986; 156: 37-48.
14. 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
15. TANAKA M, TANAKA T, HARATA M, SUZUKI T, MITSUI Y: Triplet repeat-containing ribosomal protein L14 gene in immortalized human endothelial cell line (t-HUE4). *Biochem Biophys Res Commun* 1998; 243: 531-7.
16. GUAN KL,DIXON JE: Eukaryotic proteins expressed in *Escherichia coli*: An improved thrombin cleavage and purification procedure of fusion proteins with glutathione S-transferase. *Anal Biochem* 1991; 192: 262-7.
17. SMITH DB, JOHNSON KS: Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene* 1988; 67: 31-40.
18. SCHETT G, STEINER G, SMOLEN JS: Nuclear antigen histone H1 is primarily involved in lupus erythematosus cell formation. *Arthritis Rheum* 1998; 41: 1446-55.
19. CHAN YL, OLVERA J, WOOL IG: The primary structure of rat ribosomal protein L14. *Biochem Biophys Res Commun* 1996 15; 222: 427-31.
20. SATO T, UCHIUMI T, ARAKAWA M, KOMINAMI R: Serological association of lupus autoantibodies to a limited functional domain of 28S ribosomal RNA and to the ribosomal proteins bound to the domain. *Clin Exp Immunol* 1994; 98: 35-9.
21. HASEGAWA H, UCHIUMI T, SATO T, ARAKAWA M, KOMINAMI R: Anti-Sm autoantibodies cross-react with ribosomal protein S10: a common structural unit shared by the small nuclear RNP proteins and the ribosomal protein. *Arthritis Rheum* 1998; 41: 1040-6.
22. HASEGAWA H, UCHIUMI T, SATO T, *et al.*: High frequency of antibody activity against ribosomal protein S10 in anti-Sm sera from patients with systemic lupus erythematosus. *Lupus* 1999; 8: 439-43.
23. TSUZAKA K, LEU AK, FRANK MB, *et al.*: Lupus autoantibodies to double-stranded DNA cross-react with ribosomal protein S1. *J Immunol* 1996; 156: 1668-75.
24. DOENECKE D, TONJES R: Differential distribution of lysine and arginine residues in the closely related histones H1 and H5. Analysis of a human H1 gene. *J Mol Biol* 1986; 187: 461-4.
25. LINDNER H, SARG B, HOERTNAGL B, HELLIGER W: The microheterogeneity of the Mammalian H10 Histone evidence for an age-dependent deamidation. *J Biol Chem* 1998; 273: 13324-30.
26. COHEN MG, POLLARD KM, WEBB J: Antibodies to histones in systemic lupus erythematosus:prevalence, specificity, and relationship to clinical and laboratory features. *Ann Rheum Dis* 1992; 51 :61-6.
27. SHRIVER SP, SHRIVER MD, TIRPAK DL, *et al.*: Trinucleotide repeat length variation in the human ribosomal protein L14 gene (RPL14): Localization to 3p21.3 and loss of heterozygosity in lung and oral cancers. *Mutat Res* 1998; 406: 9-23.
28. YOKOHARI R, TSUNEMATSU T: Application, to Japanese patients, of the 1982 American Rheumatism Association revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1985; 28: 693-8.
29. HASHIMOTO H, NISHIMURA Y, DONG RP, *et al.*: HLA antigens in Japanese patients with systemic lupus erythematosus. *Scand J Rheum* 1994; 23: 191-6.