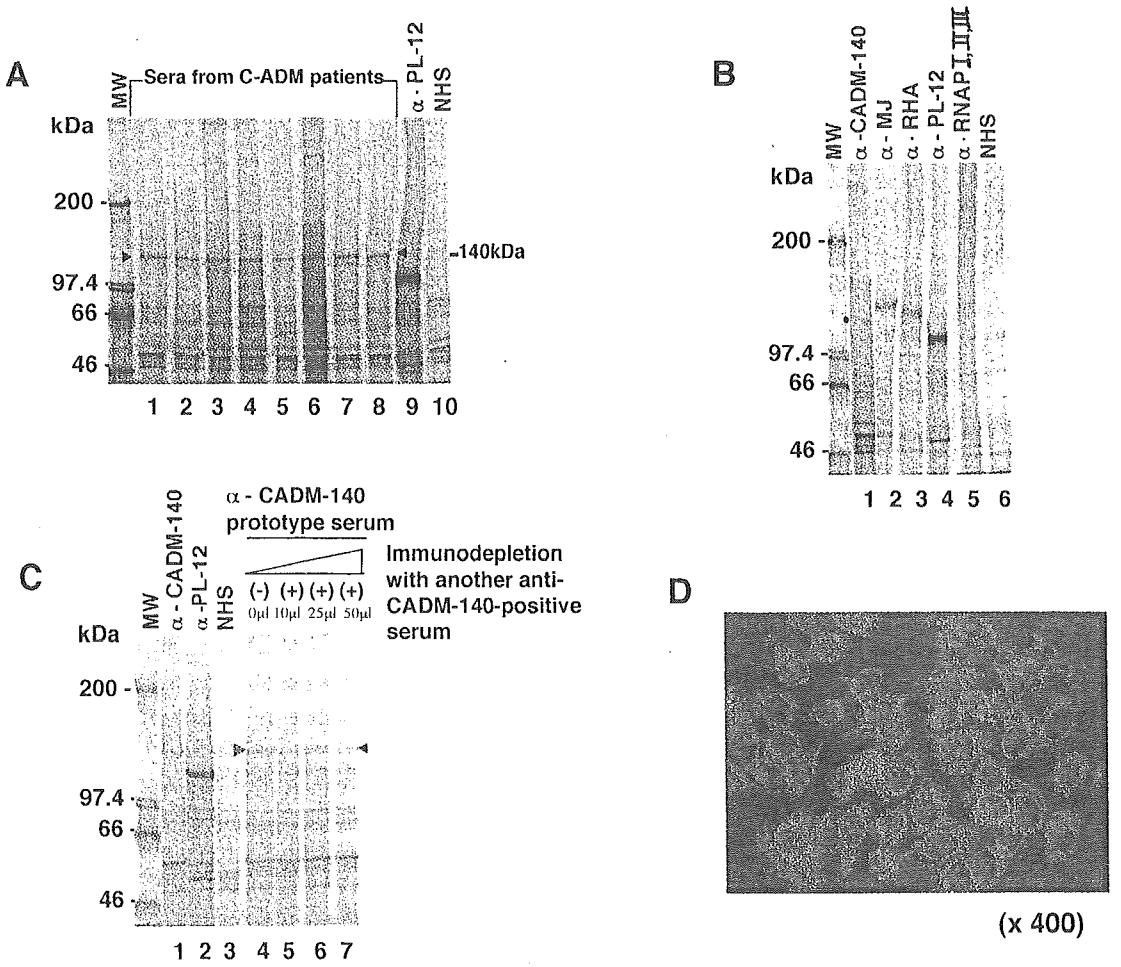


Figure 1. A, Immunoprecipitation of polypeptides with sera from patients with clinically amyopathic dermatomyositis (C-ADM), using ^{35}S-methionine–labeled K562 cell extracts. Lanes 1–8, Sera from C-ADM patients; lane 9, anti–PL-12 serum; lane 10, control normal human serum (NHS). A 140-kd protein was recognized by 8 sera from C-ADM patients (lanes 1–8). **B,** Immunoprecipitation of polypeptides by the prototype serum and by other known autoantibodies. Lane 1, The prototype anti–CADM-140 serum; lane 2, anti-MJ serum; lane 3, anti–RNA helicase A (RHA) serum; lane 4, anti–PL-12 (alanyl–transfer RNA synthetase) serum; lane 5, anti–RNA polymerase I, II, and III (RNAP I, II, and III) serum; lane 6, control NHS. Anti–CADM-140 serum immunoprecipitated an ~140-kd polypeptide that was easily distinguished from that of other known antibodies. **C,** Immunodepletion studies. Sera used for immunoprecipitation were as follows: lane 1, anti–CADM-140; lane 2, anti–PL-12; lane 3, control NHS; lanes 4–7, immunoprecipitation with anti–CADM-140 serum after absorption by another anti–CADM-140–positive serum in a dose-dependent manner. **Arrows** in **A** and **C** denote the 140-kd polypeptide. The sizes of the molecular weight markers are indicated to the left in **A–C. D,** Immunofluorescence pattern of HEp-2 cells stained with anti–CADM-140 serum. A granular or reticular cytoplasmic staining pattern on HEp-2 cells was observed. (Original magnification × 400.)

anti–PL-12 antibody (Figure 1B, lane 4). These results clearly indicate that the 140-kd polypeptide immunoprecipitated by the prototype serum was different from the proteins immunoprecipitated by these other known antibodies. We designated this new autoantibody specificity as anti–CADM-140.

The prototype serum depleted extracts of the 140-kd polypeptide in a dose-dependent manner (Figure 1C, lanes 4–7), and the polypeptide recognized by the prototype serum was no longer immunoprecipitated in these extracts (Figure 1C, lane 7). In contrast, the depletion of radiolabeled K562 cell extracts with the use of autoantibodies of different immunologic specificities did not affect the levels of the anti–CADM-140–specific antigen (results not shown). When sera positive for anti–CADM-140 antibodies were assessed in indirect IF studies, a granular or reticular cytoplasmic staining pattern was observed (Figure 1D).

Table 1. The frequencies of myositis-specific, myositis-associated, and anti–CADM-140 antibodies in patients with connective tissue diseases and IPF*

Autoantibodies	PM (n = 61)	DM (n = 42)		RA (n = 50)	SLE (n = 46)	MCTD/OL (n = 27)	Systemic sclerosis (n = 22)	Sjögren's syndrome (n = 7)	IPF (n = 43)
		Classic DM (n = 27)	C-ADM (n = 15)						
Myositis-specific									
Anti-ARS (anti–Jo-1)	10 (16)	6 (22)	0	0	0	0	0	0	0
Anti-ARS (non–anti–Jo-1)	10 (16)	2 (7)	0	0	0	1 (4)	0	0	4 (9)
Anti-SRP	5 (8)	0	0	0	0	0	0	0	0
Anti–Mi-2	0	2 (7)	0	0	0	0	0	0	0
Myositis-associated									
Anti-SSA/Ro	3 (5)	3 (11)	2 (14)	8 (16)	15 (33)	6 (22)	1 (5)	5 (71)	1 (2)
Anti–U1 RNP	2 (3)	2 (7)	0	1 (2)	18 (39)	23 (85)	2 (9)	0	0
Anti–CADM-140	0	0	8 (53)	0	0	0	0	0	0

* Values are the number (%) of patients. Anti-PM/Scl and other myositis-associated autoantibodies were not detected in any of the sera tested. PM = polymyositis; DM = dermatomyositis; C-ADM = clinically amyopathic dermatomyositis; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; MCTD/OL = mixed connective tissue disease/overlap syndrome; IPF = idiopathic pulmonary fibrosis; anti-ARS = anti–aminoacyl–transfer RNA synthetase; anti-SRP = anti–signal recognition particle.

Disease specificity of the anti–CADM-140 antibodies. The frequencies of myositis-specific antibodies, myositis-associated antibodies, and anti–CADM-140 antibodies are summarized in Table 1. Myositis-specific antibodies are found in most patients with myositis, whereas myositis-associated antibodies are frequently found in patients without myositis (12). Among the patients with CTDs or IPF, myositis-specific antibodies (33 with anti-ARS, 5 with anti-SRP, 2 with anti–Mi-2) and myositis-associated antibodies (44 with anti-SSA/Ro, 48 with anti–U1 RNP, none with anti-PM/Scl or other myositis-associated antibodies) were detected. Anti–CADM-140 autoantibodies were found in 19% of sera from patients with DM (especially in 53% with the C-ADM subtype), but were not detected in patients with other CTDs or IPF.

Clinical features of C-ADM patients with anti–CADM-140. Clinical findings were compared between DM patients (including those with C-ADM) with anti–CADM-140 autoantibodies and those without anti–CADM-140 autoantibodies (Table 2). There were no significant differences in the frequencies of skin symptoms. However, the frequency of rapidly progressive ILD was significantly increased in anti–CADM-140–positive patients compared with that in anti–CADM-140–negative patients (50% versus 6%; $P = 0.008$). No myositis-specific antibodies were found in patients with anti–CADM-140; nevertheless, there was no significant

Table 2. Comparison of clinical features in anti–CADM-140–positive versus anti–CADM-140–negative patients with dermatomyositis

Feature	Anti–CADM-140–positive (n = 8)	Anti–CADM-140–negative (n = 34)	*P*
Age at onset, mean ± SD years	44.5 ± 12.7	46.5 ± 15.7	NS
No. male/no. female	2/6	8/26	NS
Gottron's sign or papules	75	88	NS
Heliotrope rash	50	53	NS
Muscle weakness	0	76	0.02
Elevation of CK	25	74	0.03
Fever	25	50	NS
Raynaud's phenomenon	13	24	NS
Arthritis	50	71	NS
Rapidly progressive ILD	50	6	0.008
Malignancy	0	18	NS
MSAs	0	29	NS
MAAs	13	18	NS

* Except where indicated otherwise, values are the percent of patients. NS = not significant; CK = creatine kinase; ILD = interstitial lung disease; MSAs = myositis-specific autoantibodies; MAAs = myositis-associated autoantibodies.

difference in the frequency of these autoantibodies in comparison with the anti–CADM-140–negative group.

None of the 8 patients with anti–CADM-140–positive sera were treated with steroids or other immunosuppressive medications prior to being assessed for C-ADM. All of these patients had Gottron's sign or papules, or periorbital heliotrope erythema and skin biopsy specimens yielding results compatible with DM. None of these patients had muscle weakness. CK levels were in the normal range in 6 patients (75%) and slightly elevated in the remaining 2 patients. Of the 6 patients assessed for the muscle enzyme aldolase, levels were normal in 2 patients. Of the 2 patients who underwent muscle MRI, neither showed findings compatible with a diagnosis of myopathy. Four patients had a muscle biopsy, and 2 of the muscle specimens exhibited mild infiltration of inflammatory cells, but there was no evidence of necrosis of muscle fibers, variation in fiber size, regeneration, or phagocytosis. Of the 7 patients with ILD (88%), 4 developed rapidly progressive disease.

DISCUSSION

We have identified novel autoantibodies (anti–CADM-140 autoantibodies) to an ~140-kd polypeptide in patients with DM. Anti–CADM-140 antibodies were detected specifically in patients with DM, especially those with C-ADM. In addition, anti–CADM-140 antibodies were associated with rapidly progressive ILD.

It has been reported that amyopathic DM may be accompanied by rapidly progressive ILD, especially in Japanese patients and other Asian patients (7). In contrast, rapidly progressive ILD was shown to be rare in patients with amyopathic DM in a North American population (13). In our series, 5 of 15 patients with C-ADM (33%) (4 of whom had anti–CADM-140 antibodies) had rapidly progressive ILD during their clinical course. Rapidly progressive ILD was more frequent in our series compared with that reported previously in North American populations (13). Although the number of patients that we studied was very limited, it remains possible that racial differences are the reason for this discrepancy, because other clinical studies of Japanese patients also demonstrated findings similar to ours (7).

Furthermore, in a recent preliminary report, using immunoprecipitation and immunoblotting of HeLa cell extracts, Targoff et al documented the presence of antibodies to a 155-kd protein and/or Se protein in patients with C-ADM (8). Thirteen of 18 C-ADM sera possessed an anti–155-kd polypeptide antibody, and 6 also immunoprecipitated a 95-kd polypeptide (anti-Se antibody). In contrast, Oddis et al identified the anti-MJ antibody, which was also found to recognize a 140-kd polypeptide, in patients with juvenile DM (14,15). We have been able to conclude that anti–CADM-140 is distinctively different from anti-MJ, because the molecular weights of the immunoprecipitated polypeptides are different. Moreover, the clinical features of anti-MJ are quite different from those associated with anti–CADM-140. Anti-MJ is detected mainly in juvenile DM, has been observed in the US and Argentina, and is clinically characterized by severe DM with a chronic and polycyclic course, sometimes accompanied by vasculitis (14). In order to elucidate the racial differences in the frequency of these antibodies, the examination of a larger number of patients from several different populations is required.

Our results have thus demonstrated the presence of anti–CADM-140 autoantibodies in patients with C-ADM, and these were found to be associated with rapidly progressive ILD. Further studies of this novel autoantibody specificity may provide insight into the pathogenic mechanisms of C-ADM accompanied by rapidly progressive ILD.

ACKNOWLEDGMENTS

We thank Ms N. Fertig for preparing the prototype anti-MJ serum, and Ms Mutsuko Ishida for assisting in RNA immunoprecipitation assays.

REFERENCES

1. Plotz PH, Rider LG, Targoff IN, Raben N, O'Hanlon TP, Miller FW. Myositis: immunologic contributions to understanding cause, pathogenesis, and therapy. Ann Intern Med 1995;122:715–24.
2. Pearson CM. Polymyositis and dermatomyositis. In: Koopman WJ, editor. Arthritis and allied conditions. Baltimore: Williams & Wilkins; 1997. p. 742–61.
3. Hirakata M, Mimori T, Akizuki M, Craft J, Hardin JA, Homma M. Autoantibodies to small nuclear and cytoplasmic ribonucleoproteins in Japanese patients with inflammatory muscle disease. Arthritis Rheum 1992;35:449–56.
4. Targoff IN, Johnson AE, Miller FW. Antibody to signal recognition particle in polymyositis. Arthritis Rheum 1990;33:1361–70.
5. Targoff IN, Reichlin M. The association between Mi-2 antibodies and dermatomyositis. Arthritis Rheum 1985;28:796–803.
6. Sontheimer RD. Would a new name hasten the acceptance of amyopathic dermatomyositis (dermatomyositis sine myositis) as a distinctive subset within the idiopathic inflammatory dermatomyopathies spectrum of clinical illness? J Am Acad Dermatol 2002;46:626–36.
7. Tokiyama K, Tagawa H, Yokota E, Nagasawa K, Kusaba T, TsudaY, et al. Two cases of amyopathic dermatomyositis with fatal rapidly progressive interstitial pneumonitis. Ryumachi 1990;30: 204–11. In Japanese.
8. Targoff IN, Trieu EP, Sontheimer RD. Autoantibodies to 155 kD

and Se antigens in patients with clinically-amyopathic dermatomyositis [abstract]. Arthritis Rheum 2000;43 Suppl 9:S194.
9. Hirakata M, Suwa A, Nagai S, Kron MA, Trieu EP, Mimori T, et al. Anti-KS: identification of autoantibodies to asparaginyl-transfer RNA synthetase associated with interstitial lung disease. J Immunol 1999;162:2315–20.
10. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. Proc Natl Acad Sci U S A 1979;76: 4350–4.
11. Lane RJ, Emslie-Smith A, Mosquera IE, Hudgson P. Clinical, biochemical and histological responses to treatment in polymyositis: a prospective study. J R Soc Med 1989;82:333–8.
12. Targoff IN. Laboratory testing in the diagnosis and management of idiopathic inflammatory myopathies. Rheum Dis Clin North Am 2002;28:859–90.
13. Euwer RL, Sontheimer RD. Amyopathic dermatomyositis: a review. J Invest Dermatol 1993;100:124S–7S.
14. Oddis CV, Fertig N, Goel A, Espada G, Confalone Gregorian M, Maldonado Cocco JA, et al. Clinical and serological characterization of the anti-MJ antibody in childhood myositis [abstract]. Arthritis Rheum 1997;40 Suppl 9:S139.
15. Espada G, Confalone Gregorian M, Ortiz Z, Fertig N, Londino AV, Oddis CV, et al. Serum autoantibodies in juvenile idiopathic inflammatory myopathies (IIM) in a cohort of Argentine patients [abstract]. Arthritis Rheum 1997;40 Suppl 9:S140.

ARTHRITIS & RHEUMATISM
Vol. 52, No. 2, February 2005, pp 668–669

CONCISE COMMUNICATION

DOI 10.1002/art.20766

Association between autoantibodies to the Ku protein and DPB1*

The Ku protein, a heterodimer consisting of 70-kd (p70) and 80-kd (p80) polypeptide subunits, binds free ends of double-stranded DNA (dsDNA). Once associated with DNA it creates a binding site for the catalytic subunit of the holoenzyme known as DNA-dependent protein kinase. This enzyme is essential for repairing dsDNA breaks that occur during radiation injury and V(D)J recombination (1).

Autoantibodies to the Ku protein were identified originally in 9 individuals among a randomly selected group of 330 Japanese patients (3%) with various connective tissue diseases studied with a classic immunodiffusion assay. Six of the patients who tested positive for autoantibodies came from a subgroup of 11 individuals (55%) with polymyositis–scleroderma (PM–scleroderma) overlap syndrome (2).

A somewhat different picture of anti-Ku autoantibodies emerged from studies of patients in the US. Reeves observed anti-Ku autoantibodies in the sera of 39% of patients with systemic lupus erythematosus (SLE), 55% of patients with mixed connective tissue disease, and 40% of patients with scleroderma, using an enzyme-linked immunosorbent assay (3). These antibodies also appear to be much more common among African American patients than white patients with SLE (4). Using immunoprecipitation assays, Francoeur et al observed anti-Ku antibodies in 10% of patients with SLE and in no samples obtained from patients with scleroderma (5). These observations suggest that anti-Ku antibodies have unique clinical associations in different racial groups, but further studies applying the same assay systems to different populations simultaneously will be required to confirm this speculation.

In the last several years, it has become clear that autoantibodies to nucleoproteins are antigen driven and require T helper cell support. Therefore, variations of autoantibody correlations in different patient groups seem likely to reflect racial differences in distribution of major histocompatability complex (MHC) phenotypes and the pattern of peptide antigens that are presented to T cells. We have now explored this idea through a genotypic analysis of all patients with anti-Ku autoantibodies at our institution in Japan.

A total of 750 Japanese patients were screened for autoantibodies in a radioimmunoprecipitation assay (6), and 21 were found to have anti-Ku autoantibodies. The presence of these antibodies was confirmed in an immunoblot assay using extracts of HeLa cells. The clinical diagnosis was established from a review of the medical record (Table 1). None of these patients had familial relationships. Clinically, 13 patients had PM or overlap syndromes with myositis (5 had PM–scleroderma, 4 had PM–scleroderma–SLE overlap, 2 had PM–SLE overlap, and 2 had PM), 5 had SLE, 2 had autoimmune hepatitis, and 1 had scleroderma according to established classification criteria (7–10). Forty-six healthy unrelated Japanese individuals served as control subjects. The HLA class II (DRB1, DQA1, DQB1, and DPB1) alleles were identified from restriction fragment length polymorphisms of polymerase chain reaction–amplified genomic DNA (11).

The HLA class II genotypes of all 21 patients are shown in Table 1. DRB1*0901 (62% of subjects versus 28% of controls; $P = 0.009$, odds ratio [OR] = 4.1), DQA1*0302 (62% versus 59%), and DQB1*0303 (62% versus 30%) were elevated in the study group, but none of these associations were statistically significant. However, DPB1*0501 was present in all patients with anti-Ku autoantibodies, compared with 59% of control subjects. This association was significant ($P = 0.0016$, OR 30) and remained significant ($P = 0.03$) when corrected for the number of alleles examined. Thirteen of the 21 patients (62%) with anti-Ku antibodies had myositis. Ten of these individuals (77%) had the class II haplotype of DRB1*0901-DQA1*0302-DQB1*0303, compared with 38% of anti-Ku–positive patients without myositis and 28% of controls ($P = 0.004$, OR 8.5). Four patients were homozygous for DRB1*0901, DQA1*0302, and DQB1*0303, but we found no indication of more severe disease in this group.

Studies of HLA associations with anti-Ku autoantibodies are limited. Yaneva and Arnett reported that the HLA class II antigen DQw1 was present in 17 of 19 anti-Ku positive patients (89%), compared with its frequency in local white (58%) and African American (61%) controls ($P = 0.01$, relative risk 5.8) (12). Although this allele occurs at increased frequency in patients with SLE, it is not associated with myositis and scleroderma. In the present study, the most striking finding is the universal occurrence of DPB1*0501 in 21 consecutive patients with anti-Ku autoantibodies. The DRB1*0901-DQA1*0302-DQB1*0303 haplotype also correlates with myositis in this patient cohort. Both DPB1*0501 and the DRB1*0901-DQA1*0302-DQB1*0303 haplotype are more common in the Japanese population than in the white population (13). It should be noted that DPB1*0501 is also a risk factor for Graves' disease in Japan (14). These findings suggest that there is a common immunogenetic background for Graves' disease and the anti-Ku autoimmune response. Therefore, these associations help to rationalize the earlier findings that anti-Ku autoantibodies are more clearly associated with myositis among the Japanese population.

Among the patients studied here, 9 had PM–scleroderma overlap syndrome with anti-Ku antibodies but none had the anti–PM-Scl, specificity. In the US population, ~10% of patients with this syndrome develop anti–PM-Scl. We have examined >100 patients with this overlap syndrome, but none have had anti–PM-Scl, nor have any of the >3,000 patients screened in our clinical diagnostic laboratory. Therefore we believe this autoantibody is rare among Japanese individuals. An explanation may be that anti–PM-Scl antibodies have been linked with DR3, a phenotype that is uncommon in the Japanese population (13). In any case, the MHC phenotype appears to exert a stronger influence over expression of specific autoantibodies than over the emergence of individual autoimmune syndromes. Further studies including analysis of MHC-restricted T cell responses could provide important clues for understanding mechanisms of onset of the PM–scleroderma overlap syndrome and the expression of anti-Ku antibodies.

Supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology, Japanese government, and from the Keio University School of Medicine.

Table 1. HLA class II genes in Japanese patients with anti-Ku autoantibodies*

Patient no.	Diagnosis	DRB1*	DQA1*	DQB1*	DPB1*
1	PM/SSc	0405/1101	0303/0505	0401/0301	0501/0402
2	PM/SSc	0901/080302	0302/0103	0303/0601	0501/0202
3	PM/SSc	0901/080302	0302/0103	0303/0601	0501/0201
4	PM/SSc	0901/0405	0302/0303	0303/0401	0201/0501
5	PM/SSc	0901/0901	0302/0302	0303/0303	0501/0402
6	PM/SSc/SLE	0901/0901	0302/0302	0303/0303	0501/0402
7	PM/SSc/SLE	0901/1401	0302/0104	0303/0503	0501/0201
8	PM/SSc/SLE	0901/1502	0302/0103	0303/0601	0501/0901
9	PM/SSc/SLE	0901/0901	0302/0302	0303/0303	0501/0201
10	PM/SLE	0901/0901	0302/0302	0303/0303	0501/0201
11	PM/SLE	0405/0405	0303/0303	0401/0401	0501/0301
12	PM	0901/0802	0302/030101	0303/0302	0501/4101
13	PM	0405/1502	0303/0103	0401/0601	0501/0901
14	SLE	0901/1501	0302/0102	0303/0602	0501/0501
15	SLE	1501/0802	0401/0102	0302/0602	0201/0501
16	SLE	0405/080302	0303/0103	0401/0601	0501/0501
17	SLE	0901/080302	0302/0103	0303/0601	0501/0201
18	SLE	080302/1302	0103/0102	0601/0604	0501/0401
19	SSc	0405/0405	0303/0303	0401/0401	0501/0201
20	AIH	0802/0802	030101/030101	0302/0302	0201/0501
21	AIH	0901/0802	0302/030101	0303/0302	0501/0501

* PM = polymyositis; SSc = systemic sclerosis (scleroderma); SLE = systemic lupus erythematosus; AIH = autoimmune hepatitis.

Michito Hirakata, MD
Akira Suwa, MD
Masataka Kuwana, MD
Shinji Sato, MD
Keio University School of Medicine
Tokyo, Japan
Tsuneyo Mimori, MD
Kyoto University
Kyoto, Japan
John A. Hardin, MD
Albert Einstein College of Medicine
Bronx, NY

1. Smider V, Rathmell WK, Lieber MR, Chu G. Restoration of x-ray resistance and V (D)J recombination in mutant cells by Ku cDNA. Science 1994;266:288–91.
2. Mimori T, Akizuki M, Yamagata H, Inada S, Yoshida S, Homma M. Characterization of a high molecular weight acidic nuclear protein recognized by autoantibodies in sera from patients with polymyositis-scleroderma overlap. J Clin Invest 1981;68:611–20.
3. Reeves WH. Use of monoclonal antibodies for the characterization of novel DNA-binding proteins recognized by human autoimmune sera. J Exp Med 1985;161:18–39.
4. Wang J, Satoh M, Kabir F, Shaw M, Domingo MA, Mansoor R, et al. Increased prevalence of autoantibodies to Ku antigen in African American versus white patients with systemic lupus erythematosus. Arthritis Rheum 2001;44:2367–70.
5. Francoeur AM, Peebles CL, Gompper PT, Tan EM. Identification of Ki (Ku, p70/p80) autoantigens and analysis of anti-Ki autoantibody reactivity. J Immunol 1986;136:1648–53.
6. Hirakata M, Mimori T, Akizuki M, Craft J, Hardin JA, Homma M. Autoantibodies to small nuclear and cytoplasmic ribonucleoproteins in Japanese patients with inflammatory muscle disease. Arthritis Rheum 1992;35:449–56.
7. Bohan A, Peter JB. Polymyositis and dermatomyositis. N Engl J Med 1975;292:344–7.
8. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.
9. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581–90.
10. Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Caucado EL, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31:929–38.
11. Inoko H, Ota M. PCR-RFLP. In: J. Bidwell, KM Hui, editors. Handbook for HLA tissue-typing techniques. Boca Raton (FL): CRC Press; 1993. p. 9–70.
12. Yaneva M, Arnett FC. Antibodies against Ku protein in sera from patients with autoimmune diseases. Clin Exp Immunol 1989;76: 366–72.
13. Imanishi T, Akaza T, Kimura A, Tokunaga K, Gojobori T. Allele and haplotype frequencies for HLA and complement loci in various ethnic groups. In: Tsuji K, Aizawa M, Sasazuki T, editors. HLA 1991: proceedings of the Eleventh International Histocompatibility Workshop and Conference. Oxford: Oxford University Press; 1992. p. 1066–222.
14. Dong RP, Kimura A, Okubo R, Shinagawa H, Tamai H, Nishimura Y, et al. HLA-A and DPB1 loci confer susceptibility to Graves' disease. Hum Immunol 1992;35:165–72.

Successful treatment of recurrent intracardiac thrombus in Behçet's disease with immunosuppressive therapy

Y. Kaneko, K. Tanaka, A. Yoshizawa, H. Yasuoka, A. Suwa, T. Satoh, S. Iwanaga, S. Ogawa, Y. Ikeda, M. Hirakata

Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan.

Yuko Kaneko, MD, Assistant; Karin Tanaka, MD, Resident; Akihiro Yoshizawa, MD, Post-graduate; Hidekata Yasuoka, MD, Assistant; Akira Suwa, MD, Assistant Professor; Toru Satoh, MD, Assistant Professor; Shiro Iwanaga, MD, Assistant Professor; Satoshi Ogawa, MD, Professor; Yasuo Ikeda, MD, Professor; Michito Hirakata, MD, Assistant Professor;

Please address correspondence and reprint requests to: Yuko Kaneko, MD, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: ykaneko@sc.itc.keio.ac.jp

Received on October 20, 2003; accepted in revised form on June 14, 2005.

ABSTRACT

Behçet's disease (BD) is a chronic multisystem inflammatory disorder characterized by recurrent oral and genital ulcers, skin eruptions and uveitis. Neurological, gastrointestinal, and musculoskeletal systems are also involved. Although venous and arterial vasculitis occur in up to one-third of patients, intracardiac thrombus is a very rare complication. We herein report the case of a 46-year-old man with BD who presented with a large right atrial thrombus. Within a month after surgical removal, the thrombus recurred and was successfully treated with immunosuppressants that included prednisolone and cyclophosphamide.

Introduction

Behçet's disease (BD) is a chronic multisystem inflammatory disease of unknown etiology that is especially prevalent in Turkey, other Mediterranean regions and Japan (1). It is clinically characterized by inflammatory ocular involvement, recurrent oral ulcers, genital ulcers, and skin eruptions. Joints, gastrointestinal, nervous, respiratory, and vascular systems may also be involved, though cardiac involvement is infrequent (1-3). While venous thrombosis reportedly occurs in about 25% of patients, intracardiac thrombosis is extremely rare, but serious (4).

We herein describe a patient with BD who developed a recurrent intracardiac thrombus that responded to immunosuppressive therapy.

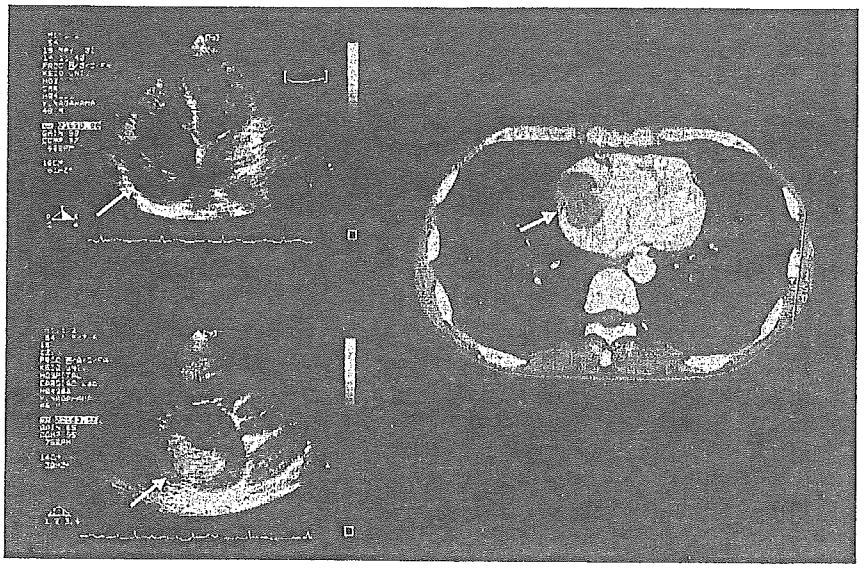
Case report

A 46-year-old Japanese man with BD was admitted to our hospital in August 2001 with recurrence of intracardiac thrombus. A year earlier, he had suffered from recurrent orogenital ulcers and erythema nodosa. In September 2000, bilateral painful swelling in his legs had appeared. He visited another hospital, where he was diagnosed as having deep vein thrombosis and anticoagulant therapy was started. At that time, echocardiography showed no cardiac mass. In January 2001, he was admitted to that hospital because of a high-grade fever over two months and exacerbation of his leg swelling. Echocardiography revealed a large mass in the right atrium, which was thought to be a thrombus because repeated blood cultures, serological examinations and the form of the mass provided no evidence of infectious endocarditis, tuberculosis or malignant disease. In addition, ulcers were detected in the terminal ileum by colonoscopy. Although the fever and the leg swelling due to thrombophlebitis had improved with continuous intravenous heparin, his intracardiac thrombus remained. The patient was admitted to our hospital in May 2001. Echocardiography and chest CT showed the homogeneous, well-defined and mobile mass (70x60 mm) on the lateral wall of the right atrium (Fig. 1). The patient was diagnosed as BD based on the criteria of the international study group (3). There were no active symptoms other than thrombus, therefore steroid therapy had not been considered. Because the thrombus was large and failed to respond to anticoagulant therapy, and multiple pulmonary thromboembolisms were found by chest CT with dynamic contrast enhancement, thrombectomy was performed in June. After warfarin therapy was started, he was discharged in July. A month later, he was readmitted for the asymptomatic recurrence of the intracardiac thrombus.

On physical examination, the patient's blood pressure, pulse rate, and body temperature were 94/70 mmHg, 60/minute, and 36.2°C, respectively. He was noted as having oral ulcers and a 3/6 holosystolic murmur at the apex.

Laboratory tests showed erythrocyte sedimentation rate (ESR) of 48 mm/h, no abnormality of urinalysis test and positive stool occult blood test; hemoglobin concentration of 10.2 g/dl, TT-INR of 2.2, FDP of 161 ng/ml (normal < 100), D-D dimer of 1.1μg/ml (normal < 1.0), C-reactive protein of 1.3 mg/dl, and normal level of protein C, protein S, and thrombomodulin. Antinuclear antibody and anti-cardiolipin antibody were negative. HLA-B51 and pathergy test were negative. His ophtamological findings were unremarkable.

The chest X-ray and ECG were normal. Echocardiography revealed a thrombus (32x17 mm) in the right atrium. A week



(a) (b)

Fig. 1. Echocardiography and chest CT before the operation. (a) Echocardiography and (b) chest CT revealed the presence of a homogeneous, well-defined and mobile mass (70 x 60 mm) on the lateral wall of the right atrium (→).

later after admission, he complained of diplopia with the right oculomotor nerve palsey and brain MRI revealed an enhanced region in the pons, suggesting neurological involvement. He did not have any other neurological findings or sagittal sinus thrombosis.

He was treated with heparin and prednisolone (PSL; 60 mg/day) immediately after admission in August 2001, and his thrombus reduced. But because it enlarged again in October, cyclophosphamide (CPA; 150mg/day) was started. In September the thrombus disappeared and he was free of any neurological manifestations. Because he developed some complications such as an untreatable lung abscess, compression fracture of the lumbar spine, and Pneumocistis jiroveci pneumonitis due to immunosuppressive therapy and high dose use of heparin for a long period, CPA was discontinued, PSL dose was reduced, and warfarinization was started. CPA was restarted in 2003. He has been well over two years without recurrence of the intracardiac thrombus.

Discussion

BD is a multisystem inflammatory disorder of unknown etiology. Cardiac manifestations include pericarditis, myocarditis, endocarditis, conduction-system abnormalities, valvular regurgitaion, and coronary arteritis (1, 2). However, intracardiac thrombosis is extremely rare (4), and the treatment is still controversial (5).

Histopathological examination of biopsy and surgical specimens are helpful in determining the pathological features of cardiac lesions. Mogulkoc *et al.* reviewed 25 BD patients with intracardiac thrombus and reported the presence of endomyocarditis, fibrosis, and inflammatory cell infiltrates in some of specimens (5). We were unable to obtain an endomyocardial specimen because doing so would have increased the risk of endothelial injury and pulmonary thromboembolism.

Thrombus formation in BD probably occurs by endothelial cell ischemia or disruption which leads to an enhanced platelet aggregation, an increase of fibrinolytic inhibitors such as plasminogen activator inhibitor (PAI-1), and a reduction of natural anticoagulants such as thrombomodulin (6, 7). It has also been reported that activated protein C resistance, an inherited coagulation defect, was more frequent in Behçet's patients, especially those with thrombosis (6, 8). The frequency of anti-phospholipid antibody is high in

Table I. Six Japanese cases of Behçet's disease with intracardiac thrombus.

Study	Sex	Age (yr)	HLA B51	Disease duration	Involved cavity	Treatment of intracardiac thrombus	Outcome (time of recurrence)
Fukuzawa *et al.*	F	72	NA	30 yr.	RA	Thrombolytic, PSL, CPA	Died
Nakata *et al.*	M	12	+	None	1st RA	Surgical removal	Recurrence (4 wk)
					2nd RA	Surgical removal	Recurrence (7 wk)
					3rd RA	PSL, LMWH	Disappearance
Yoshimura *et al.*	M	30	+	2 yr.	1st RV	Surgical removal	Recurrence (10 d)
					2nd RV	PSL, heparin, urokinase	Disappearance
Eguchi *et al.*	M	19	NA	None	1st RV	Anticoagulant	Recurrence (NA)
					2nd RV	PSL	Stable
Yasuo *et al.*	M	26	-	None	RV	Surgical removal, CyA	Stable
This case	M	46	-	6 mo.	1st RA	Surgical removal	Recurrence (4 wk)
					2nd RA	PSL, CPA, heparin	Disappearance

d: day; wk: week; mo: month; yr: year; NA: not available; RA: right atrium; RV: right ventricle; PSL: prednisolone; CyA: cyclosporin; CPA: cyclophosphamide; LMWH: low molecular weight heparin.

Behçet's patients, but there may be no correlation with the occurrence of thrombosis (9). It has been reported that the frequency of the prothrombin mutation 20210 gene, which is associated with an increased risk of venous thrombosis, is high in Behçet's patients (6) but, on the other hand, no differences were observed, (10). In this case the intracardiac thrombus did not respond to anticoagulant therapy and disappeared after PSL and CPA. It should be noted that endothelial injury rather than thrombophilic factors might play a pivotal role in pathogenesis.

The clinical and laboratory features of six Japanese patients with BD who developed intracardiac thrombi are summarized in Table I (11-15). The male to female ratio was 5:1 and mean age was 34 years (range, 12 to 72). Two of the four patients tested were positive for HLA B51. Thrombi existed in the right heart in all the patients. Various treatments have been reported, such as surgery, the use of thrombolytic agents, anticoagulants, corticosteroids and immunosuppressive agents independently or together. It should be noted that in all four patients who were not treated with corticosteroids or immunosuppressive agents, the thrombus recurred and then responded well to these medications.

The present case was BD with recurrent intracardiac thrombus. The first approach was thrombectomy, but the thrombus recurred despite the use of warfarin, which was successfully treated with heparin, PSL and CPA. Corticosteroids and immunosuppressive agents might be useful for inhibiting thrombus formation and promoting fibrynolytic effect by suppressing endothelial inflammation and injury.

In conclusion, it is worth considering the administration of PSL and/or immunosuppressive agents against intracardiac thrombus in BD. The mechanism of intracardiac thrombus in BD is still unknown and should be elucidated to establish a more specific therapy.

References

1. KAKLAMANI VG, VAIOPOULOS G, KAKLAMANI PG: Behçet's disease. *Semin Arthritis Rheum* 1998; 27: 197-217.
2. SAKANE T, TAKENO M, SUZUKI N, INABA G: Behçet's disease. *N Engl J Med* 1999; 34: 1284-91.
3. INTERNATIONAL STUDY GROUP FOR BEHÇET'S DISEASE: Criteria for diagnosis of Behçet's disease. *Lancet* 1990; 335: 1078-80.
4. KOC Y, GULLU I, AKPEK G *et al.*: Vascular involvement in Behçet's disease. *J Rheumatol* 1992; 19: 402-10.
5. MOGULKOC N, BURGESS MI, BISHOP PW: Intracardiac thrombus in Behçet's disease. *Chest* 2000; 118: 479-87.
6. LEIBA M, SIDI Y, GUR H, LEIBA A, EHRENFELD M: Behçet's disease and thrombophilia. *Ann Rheum Dis* 2001; 60: 1081-5.
7. SCHMITZ-HUEBNER U, KNOP J: Evidence for an endothelial cell dysfunction in association with Behçet's disease. *Thromb Res* 1984; 34: 277-85.
8. KOSAR A, HAZNEDAROGLU IC, BUYUKASIK Y, KIRAZLI S, DUNDAR SV: Activated protein C resistance in Behçet's disease. *Rheumatol Int* 1998; 17: 249-50.
9. MADER R, ZIV M, ADAWI M, MADER R, LAVI I: Thrombophilic facters and their relation to thromboembolic and other clinical manifestations in Behçet's disease. *J Rheumatol* 1999; 26: 2404-8.
10. ESPINOSA G, FONT J, TASSIES D *et al.*: Vascular involvement in Behçet's disease: relation with thrombophilic factors, coagulation activation, and thrombomodulin. *Am J Med* 2002; 112: 37-43
11. FUKUZAWA M, SAKAKI T, MAEJIMA T *et al.*: An autopsy case of vasculo-Behçet's disease with organized thrombosis in large veins. *Byori to Rinsho* 1993; 11: 861-5. (in Japanese)
12. NAKATA Y, AWAZU M, KOJIMA Y, TOKUMURA H, YAMAGISHI H, YAMASHITA H: Behçet's disease presenting with a right atrial vegetation. *Pediatr Cardiol* 1995; 16: 150-152.
13. YASUO M, NAGANO S, YAZAKI Y *et al.*: Pulmonary embolism due to right ventricular thrombus in case of Behçet's disease. *Jpn Circ J* 1999; 63: 909-11.
14. YOSHIMURA H, ISHII J, WATANABE N *et al.*: A case of cardiovascular Behçet's disease detected as multiple nodular shadows on chest X-ray. *Nihon Kyobu Shikkan Gakkai Zasshi* 1997; 35: 1074-9 (in Japanese).
15. EGUCHI M, NAKATA T, AOYAMA S *et al.*: A case of vascular Behcet's disease associated pulmonary infarction due to intracardiac thrombus presenting hemoptysis as a chief complaint. *Jpn Circ J* 1997; 61: Suppl. II (Abstract in Japanese).

Mod Rheumatol (2006) 16:36–38
DOI 10.1007/s10165-005-0443-5

CASE REPORT

Yuko Kaneko · Akira Suwa · Yasuo Ikeda
Michito Hirakata

Pneumocystis jiroveci pneumonia associated with low-dose methotrexate treatment for rheumatoid arthritis: report of two cases and review of the literature

Received: September 1, 2005 / Accepted: October 19, 2005

Abstract Low-dose methotrexate (MTX) therapy is widely used for rheumatoid arthritis (RA) because of its favorable efficacy and toxicity profile. Although *Pneumocystis jiroveci* pneumonia (PCP) is most often seen in severely immunosuppressed patients, PCP complicating low-dose MTX therapy for RA has been reported to sometimes occur. We herein report two cases of patients who developed PCP during treatment with low-dose MTX, and discuss the importance of prophylaxis for this opportunistic infection.

Key words Methotrexate (MTX) · *Pneumocystis jiroveci* pneumonia (PCP) · Prophylaxis · Rheumatoid arthritis (RA)

Introduction

Low-dose weekly pulse methotrexate (MTX) therapy is most commonly used for rheumatoid arthritis (RA) because of its favorable efficacy to reduce symptoms and prevent progressive structural damage.[1] However, this therapy has been recently implicated as a risk factor for opportunistic infections. *Pneumocystis jiroveci* pneumonia (PCP) is most often seen in severely immunosuppressed patients related to acquired immunodeficiency syndrome (AIDS) and treatment with strong cytotoxic agents or immunosuppressive drugs. In 1983 PCP complicating low-dose MTX therapy for RA was reported.[2] Due to the wide diversity for the options for the treatment of RA including biological agents, we should take greater care of PCP. We herein describe two patients who developed PCP during low-dose MTX therapy and discuss the importance of appropriate prophylaxis.

Y. Kaneko (✉) · A. Suwa · Y. Ikeda · M. Hirakata
Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
Tel. +81-3-3353-1211 (ext. 62315); Fax +81-3-5379-5037
e-mail: ykaneko@sc.itc.keio.ac.jp

Case reports

Patient 1

A 68-year-old woman suffering from seropositive RA for 17 years had been treated with MTX in a dosage of 5 mg/week, prednisolone (PSL) 6 mg/day, bucillamine 200 mg/day, and diclofenac 25 mg/day. After 147 months of MTX therapy, she began to complain of fever and dyspnea. On admission her body temperature was 40°C and a lung examination showed bilateral crackles. Laboratory examinations showed: white blood cells (WBC) 13200/μl (lymphocytes 660/μl), hemoglobin 14.1 g/dl, platelets 20.7 × 10^4/μl, lactate dehydrogenase (LDH) 480 IU/l, C-reactive protein (CRP) 11 mg/dl, IgG 938 mg/dl, β-D-glucan >600 pg/ml, KL-6 749 U/l. Her arterial blood gas analysis (BGA) showed type I respiratory failure (PaO_2 52 torr, $PaCO_2$ 27 torr, pH 7.57). A chest radiograph revealed bilateral ground-glass infiltrates and reticular shadows (Fig. 1). A polymerase chain reaction (PCR) assay of bronchoalveolar lavage (BAL) fluid showed *Pneumocystis* (PC). Aerobe, anaerobe, and fungal cultures of lavage fluid and cytomegalovirus (CMV) antigenemia were negative. Methotrexate was immediately discontinued, and high-dose trimethopurim sulfamethoxazole (TMP-SMX) and methylprednisolone (mPSL) pulse therapy was administered, resulting in both a clinical and radiographic improvement.

Patient 2

A 73-year-old woman suffering from malignant RA for 14 years had been treated with MTX in a dosage of 7.5 mg/week, PSL 16 mg/day, and diclofenac 75 mg/day. After 13 months of MTX therapy, she developed general malaise and gait disturbance. On admission she was afebrile and coarse crackles were audible in the left lung field. Laboratory examinations showed: WBC 4400/μl (lymphocytes 44/μl), hemoglobin 10.4 g/dl, platelets 20.7 × 10^4/μl, LDH 512 U/l, CRP 23 mg/dl, IgG 477 mg/dl, β-D-glucan 424 pg/ml. Arterial BGA revealed PaO_2 25 torr, $PaCO_2$ 42 torr, and pH

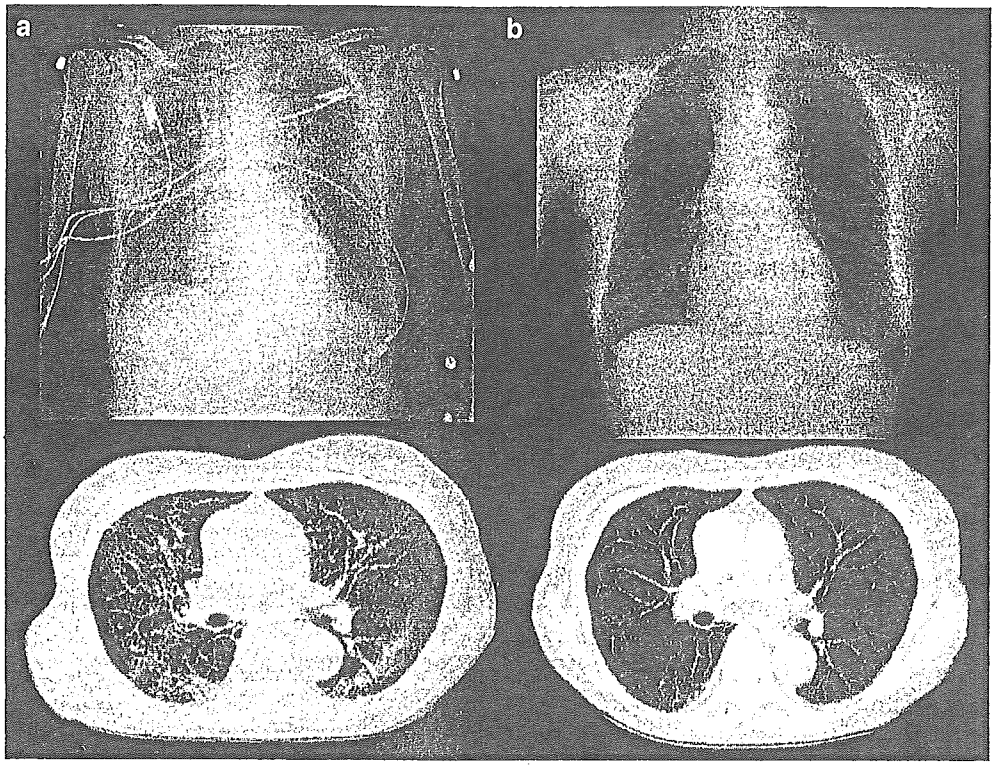


Fig. 1a,b. Chest X-ray and computed tomography. **a** Chest radiographs revealed bilateral ground-glass infiltrates and reticular shadows. **b** The shadows disappeared after treatment with prednisolone and trimethopurim/ sulfamethoxazole

7.51, and ventilation support was required. A chest radiograph revealed interstitial and alveolar infiltrations, preferentially in the left lung. Bronchoalveolar lavage fluid revealed PC by Grocotto staining. High-dose intravenous TMP-SMX was given for 7 days together with mPSL pulse therapy. TMP-SMX was changed to pentamidine isetionate, since her symptoms were complicated by severe pancytopenia due to TMP-SMX. However, she died of *Aspergillus pneumonia* and disseminated intravenous coagulation 3 weeks later. Autopsy revealed infarctions of multiple organs with intravenous thrombi in addition to diffuse fibrosis in her bilateral lungs.

Discussion

We herein reported two patients who developed PCP during low-dose MTX therapy. A good response was achieved by early diagnosis and combination therapy with TMP-SMX and mPSL in patient 1, while patient 2 was unfortunately complicated with total *Aspergillus* infection caused by a severely immunocompromised state due to concomitant PSL use, hypogammaglobulinemia, and pancytopenia. The prognosis of PCP improved with advanced treatment, but PCP often remains fatal even today.

Since the first report in 1983,[2] the occurrence of PCP during treatment with low-dose MTX in RA has attracted a great deal of attention. Formerly the PC organism was thought to be a protozoan named *Pneumocystis carinii*, but the organism was later revealed to be a fungus and not related to zoonosis by DNA analysis. As a result, it is now referred to as *Pneumocystis jiroveci*, which causes pneumonia in humans.[3] *Pneumocystis jiroveci* pneumonia is most often seen in severely immunosuppressed patients, related to AIDS and treatment with strongly cytotoxic agents or immunosuppressive drugs. The use of a PSL dose of greater than 30 mg/day was reported to be associated with a risk of developing PCP.[4] Recently, PCP complicating low-dose MTX therapy for RA has been reported to sometimes occur and it thus should be considered in the differential diagnosis for interstitial pneumonitis, including MTX-induced pneumonitis. *Pneumocystis jiroveci* pneumonia is definitely diagnosed by the detection of PC organisms in appropriate respiratory specimens using Grocotto methanamine silver staining and an immunofluorescence assay. The PCR technique has been reported to be useful for an early diagnosis. However, the asymptomatic carriage of PC has also been reported in 44% in the patients who receive corticosteroids equivalent to >20 mg/day PSL.[5] The polymerase chain reaction is superior in sensitivity to staining, but inferior in specificity.[6] Clinical findings, laboratory data, and response to treatment should be considered when confirming the diagnosis. Because the BAL fluid of patient 1 was negative in Grocotto staining but positive in PCR, we diagnosed her as having PCP by taking all factors into consideration after we started to administer PSL and TMP-SMX.

The pulmonary adverse effects associated with MTX are reported to occur in 1%–5% of cases,[7,8] and opportunistic infections, such as PCP, CMV pneumonia, disseminated herpes zoster, cryptococcosis, and widespread nocardiosis[9–11] have been found to accumulate with the increased use of MTX. Although the mechanisms by which

MTX is effective for RA are still unclear, many anti-inflammatory and immunosuppressive actions, such as the inhibition of cellular proliferation, alterations in the lymphocyte subsets, a decreased cytokine production, the suppression of T-cell activation, and cell adhesion molecules,[1-12] have all been hypothesized to play a role. The concomitant use of nonsteroidal anti-inflammatory drugs, which can raise the plasma concentration of MTX by displacing it from albumin binding sites and impairing its renal excretion, could also have potentiated the MTX toxicity.[13] Furthermore, the combination therapy of MTX and corticosteroid and/or other immunosuppressants has been suggested to be a risk factor for opportunistic infections. Prednisolone had been used in our two cases. It was interesting to note that patient 1 developed PCP while taking low-dose PSL. Lymphocytopenia may contribute to susceptibility for PCP, but PCP sometimes occurs in patients with a normal lymphocyte count.[14] In patient 1, the lymphocytes decreased at the onset of PCP, but they had been 1000–1200/μl for 3 months before the occurrence of PCP. In patient 2 the lymphocytes had been 250–300/μl for 3 months before PCP. Therefore the lymphocyte count is not considered to always be a risk factor for PCP.

Inokuma et al. reported the prevalence of PCP associated with autoimmune disease in 13 hospitals, which turned out to be 69 of 10 290 admitted patients between 1997 and 2001. Among them, 6 of 10 patients with RA were treated with MTX. It is noteworthy that all three patients who were treated with less than 10 mg/day PSL were receiving concomitant MTX, suggesting the administration of MTX to be a strong risk factor for developing PCP in RA.[15]

The clinical significance of prophylaxis for PCP remains controversial. However, because there has been a wide diversity in the treatment for RA including biological agents that are capable of inducing an immunocompromised state, we should take greater care with PCP. One case was previously reported to develop PCP after infliximab was used.[16] In 2005, the Japanese Ministry of Health, Labor and Welfare Study Group published a guideline in which PCP prophylaxis was recommended when patients of over 50 years old received either corticosteroids equivalent to >1.2 mg/kg per day PSL or corticosteroids equivalent to >0.8 mg/kg per day PSL and concomitant immunosuppressive agents, or in patients whose lymphocyte count was less than 500/μl.[15] The recommended dosage is TMP-SMX 4–8 g/week or aerosolized pentamidine isetionate 300 mg/2–4 weeks as a prophylaxis for PCP with autoimmune disease.[15] In patients infected with human immunodeficiency virus, Atovaquone or Azithromycin can also be used. Because TMP-SMX has a synergistic effect with MTX in inactivating dihydrofolate reductase and has an effect in increasing free MTX, the combination of TMP-SMX and MTX has a great risk of inducing pancytopenia.[17,18] We should therefore consider reducing the MTX dose when also using TMP-SMX or choosing other drugs, such as Azithromycin, as a prophylaxis for PCP.

In conclusion, PCP is a fatal complication that may occur in patients receiving low-dose MTX therapy for RA, and the optimal prophylaxis for PCP should be selected based on the patient's age, the severity of lymphocytopenia, or according to the concomitant use of corticosteroids and/or other immunosuppressive agents.

References

1. Cronstein BN. Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. Pharmacol Rev 2005;57:163–72.
2. Perruquet JL, Harrington TM, Davis DE. *Pneumocystis carinii* pneumonia following methotrexate therapy for rheumatoid arthritis. Arthritis Rheum 1983;26:1291–2.
3. Stringer JR, Beard CB, Miller RF, Wakefield AE. A new name (*Pneumocystis jiroveci*) for pneumocystis from humans. Emerg Infect Dis 2002;8:891–6.
4. Roblot F, Godet C, Le Moal G, Garo B, Faouzi Souala M, Dary M, et al. Analysis of underlying diseases and prognosis factors associated with *Pneumocystis carinii* pneumonia in immunocompromised HIV-negative patients. Eur J Clin Microbiol Infect Dis 2002;21:523–31.
5. Maskell NA, Waine DJ, Lindley A, Pepperall JCT, Wakefield AE, Miller RF, et al. Asymptomatic carriage of *Pneumocyctis jiroveci* in subjects undergoing bronchoscopy: a prospective study. Thorax 2003;58:594–7.
6. Flori P, Bellete B, Durand F, Raberin H, Cazorla C, Hafid J, et al. Comparison between real-time PCR, conventional PCR and different staining techniques for diagnosing *Pneumocystis jiroveci* pneumonia from bronchoalveolar lavage specimens. J Med Microbiol 2004;53:603–7.
7. Barrera P, Laan RF, van RPL, Dekhuijzen PN, Boerbooms AM, van de Putte LB. Methotrexate-related pulmonary complications in rheumatoid arthritis. Ann Rheum Dis 1994;53:434–9.
8. Hilliquin P, Renoux M, Perrot S, Puechal X, Menkes CJ. Occurrence of pulmonary complications during methotrexate therapy in rheumatoid arthritis. Br J Rheumatol 1996;35:441–5.
9. Clerc D, Brousse C, Mariette X, Bennet P, Bisson M. Cytomegalovirus pneumonia in a patient with rheumatoid arthritis treated with low dose methotrexate and prednisone. Ann Rheum Dis 1991;50:67.
10. Shiroky JB, Frost A, Skelton JD, Haegert DG, Newkirk MM, Neville C. Complications of immunosuppression associated with weekly low dose methotrexate. J Rheumatol 1991;18:1172–5.
11. LeMense GP, Sahn SA. Opportunistic infection during treatment with low dose methotrexate. Am J Respir Crit Care Med 1994; 150:258–60.
12. Johnston A, Gudjonsson JE, Sigmundsdottir H, Ludviksson BR, Valdimarsson H. The anti-inflammatory action on methotrexate is not mediated by lymphocyte apoptosis, but by the suppression of activation and adhesion molecules. Clin Immunol 2004;114:154–63.
13. Stenger AA, Houtman PM, Bruyn GA, Eggink HF, Pasma HR. Pneumocystis carinii pneumonia associated with low dose methotrexate treatment for rheumatoid arthritis. Scand J Rheumatol 1994;23:51–3.
14. Takeda Y, Tsuji T, Misumi M, Ideguchi H, Ueda A, Ohno S, et al. Pneumocystis carinii pneumonia associated with low dose methotrexate treatment for malignant rheumatoid arthritis. Rinsho Riumachi 2001;13:293–9.
15. Hashimoto H. Prophylaxis of *Pneumocystis jiroveci* pneumonia in autoimmune disease (in Japanese). In: Hashimoto H, editor. Clinical guideline. Tokyo: Japanese Ministry of Health, Labour and Welfare Study Group on complication and treatment of immune disease; 2005. p. 14–9.
16. Tai TL, O'Rourke KP, McWeeney M, Burke CM, Sheehan K, Barry M. Pneumocystis carinii pneumonia following a second infusion of infliximab. Rheumatology 2002;41:951–2.
17. Ferrazzini G, Klein J, Sulh H, Chung D, Griesbrecht E, Koren G. Interaction between trimethoprim-sulfamethoxazole and methotrexate in children with leukemia. J Pediatr 1990;117:823–6.
18. Groenendal H, Rampen FH. Methotrexate and trimethoprim-sulphamethoxazole – a potentially hazardous combination. Clin Exp Dermatol 1990;15:358–60.

Medical Practice

2005 vol. 22 no. 3 別冊

続発性(ステロイド性)骨粗鬆症のモニタリングと予防・治療

諏訪 昭・金子祐子・佐藤慎二

東京 文光堂 本郷

続発性(ステロイド性)骨粗鬆症のモニタリングと予防・治療

諏訪　昭・金子祐子・佐藤慎二
慶應義塾大学医学部内科/すわ・あきら　かねこ・ゆうこ　さとう・しんじ

はじめに

続発性骨粗鬆症は，膠原病，内分泌・代謝疾患，消化器疾患，薬剤など種々の原因による．なかでも，ステロイド(GC)性骨粗鬆症は，主要な要因であり，関節リウマチ(RA)患者では，GC剤服用により大腿骨頸部の骨密度は著明に減少し，大腿骨頸部骨折のリスクが増大する．本稿では，GC性骨粗鬆症の病態，診断，治療，予防についてまとめる．

骨リモデリングと骨粗鬆症

骨は破骨細胞による骨吸収と骨芽細胞による骨形成が繰り返し，再構築(リモデリング)される．健常時には，骨吸収と骨形成が平衡状態にあるが，骨吸収亢進か，骨形成低下により，骨量は低下する．骨代謝系調節には，破骨細胞前駆細胞と骨芽細胞の間にあるRANKLと呼ばれる情報伝達経路が重要である．骨粗鬆症は，低骨量で骨の微細構造を劣化し，その結果骨が脆くなり骨折を起こしやすい全身性の骨疾患と定義される．

RAと骨粗鬆症

RAでは，全身性骨粗鬆症に加え，傍関節性骨粗鬆症が認められる．RAでは，滑膜線維芽細胞や活性化T細胞，炎症性サイトカインによるRANKLを介した破骨細胞前駆細胞の活性化やTNF-αによる破骨細胞の分化促進が骨粗鬆症をもたらす．ADL低下による不動や日光曝露機会の減少，栄養摂取，吸収不良，性ホルモン分泌減少も骨粗鬆症を促進する．さらに，GC剤，メソトレキセート，シクロスポリンAなどの薬剤も関与する．

GC性骨粗鬆症の病態

GC剤による骨粗鬆症誘発のメカニズムの一つは，骨芽細胞のアポトーシス促進による寿命短縮や機能低下が関与する骨形成低下である．もう一つのメカニズムは，破骨細胞による骨吸収促進である．これには，腸管でのカルシウム(Ca)吸収低下や尿細管でのCa再吸収低下による副甲状腺機能亢進症や性腺機能低下に加え，破骨細胞のアポトーシス抑制による寿命延長もかかわる(図1)．

GC性骨粗鬆症の診断，検査

日本骨代謝学会により原発性骨粗鬆症の診断基準が提唱されている(表1)．本基準では，続発性骨粗鬆症を除外することとされているが，GC性骨粗鬆症の診断は本基準に準じ，治療介入にはカットオフ値を設定すべきと考える．

1. 脊椎X線検査

胸・腰椎X線像による骨折判定ならびに腰椎X線像による骨粗鬆化の判定を行う．

2. 骨量測定

二重エネルギーX線吸収測定法(DXA)は，腰椎，大腿骨頸部を含む全身の骨塩定量が可能であり，広く用いられている．得られた単位面積当たりの骨密度(BMD, g/cm^2)の若年成人平均値(YAM)に対する比率(% YAM)またはYAMに対する標準偏差値(Tスコア)で判定する．

3. 骨代謝マーカー

骨代謝マーカーは，骨形成マーカーと骨吸収マーカーに分けられる．このうち血清骨型アルカリホスファターゼ(BAP)，血清オステオカルシン(OC)，血清I型コラーゲンN末端架橋テロペプチド(NTx)，尿デオキシピリジノリン(DPD)，尿NTxが，保険収載検査である(表2)．骨代謝マーカー測定結果より，骨粗鬆症を高代謝回転型と低代謝回転型に分け，前者には骨吸収抑制薬(ビスホスホネート製剤，カルシトニン製剤，Ca製剤，イプリフラボン製剤，エストロゲン製剤，選択的エストロゲン受容体調節薬(SERM)など)を，後者には骨形成促進薬(ビタミン(Vit)K_2製剤，活性型$VitD_3$製剤など)と骨活性化薬を選択

0910-1551/05/¥100/頁/JCLS

- ステロイドは続発性骨粗鬆症の主要な原因である．
- RA では全身性および傍関節性骨粗鬆症がみられる．
- 骨吸収亢進，または骨形成低下により骨量は低下する．

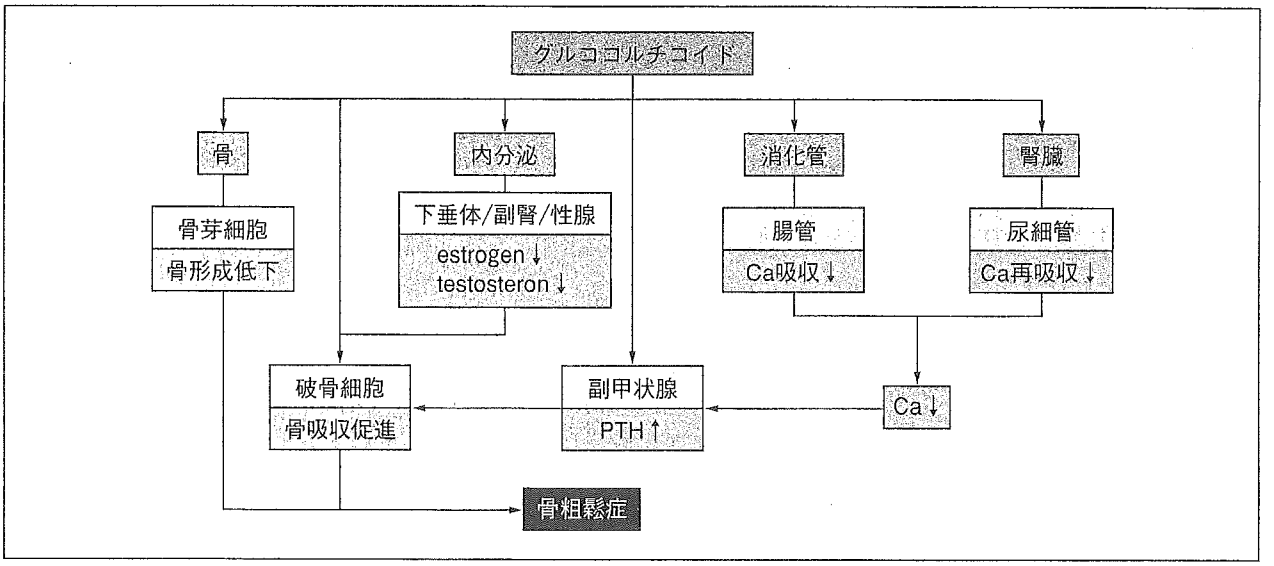


図 1　GC 性骨粗鬆症の病態

するなど，骨代謝マーカーは病態の診断，病型分類，薬剤選択の指標になる．さらに，骨代謝マーカーの基礎値からの治療後の測定値の変化率が有効最小有意変化を越える場合に，薬剤が有効と判定できる．骨代謝マーカーは，日内変動，食事の影響，性周期の影響を受けやすく注意を要する．

GC 性骨粗鬆症の予防と治療

米国リウマチ学会では，1996 年に「GC 誘発性骨粗鬆症の予防と治療のガイドライン」を作成した．その後 2001 年に改訂され，対象をプレドニゾロン 5 mg/日以上を 3 ヵ月以上服用中あるいは服用予定の患者とし，薬物療法を一次予防と二次予防に分けることが示された．危険因子の改善と VitD の基礎療法を全患者に行い，一次予防として最も効果が確立しているビスホスホネート製剤を投与することが特徴である[1]．本邦でも，GC 性骨粗鬆症に対するビスホスホネート製剤の有効性が示され[2]，ガイドラインの作成が進められている．

1.　食事療法，生活習慣の是正

本邦では Ca の成人栄養所要量 600 mg/日を下回っており，Ca の摂取を促す．禁煙または嫌煙，アルコール，コーヒーの過剰摂取を避けるよう指導する．

2.　運動療法

安静臥床や不動は，骨へのメカニカルストレスの低下や消失をきたし，全身性の廃用性骨萎縮をきたす．リハビリテーション，運動療法により，骨萎縮を抑える．

3.　薬物療法

活性型 $VitD_3$ 製剤とカルシトニン製剤には骨密度維持効果があること，ビスホスホネート製剤には高い骨密度増加効果と骨折予防効果のエビデンスがある．

a.　Ca 製剤

Ca 摂取不足者に投与し，骨量減少を予防する．保険適応は，リン酸水素 Ca と L-アスパラギン酸 Ca に限定されているが，易吸収性の乳酸 Ca が用いられる．

- 骨代謝マーカーは薬剤選択と効果判定の指標となる.
- ビスホスホネート製剤は骨量を増加させ骨折を予防する.
- ビスホスホネート製剤の妊婦への投与は禁忌である.

表1　退行期骨粗鬆症の診断基準

I. 脆弱性骨折(脊椎圧迫骨折または四肢の臨床骨折)を認める場合		
脊椎X線像で骨粗鬆化の疑いがある場合，あるいは骨塩定量が若年成人平均値(YAM)*の80%以下の症例		
II. 脆弱性骨折を認めない場合		
	脊椎X線像での骨粗鬆症化(従来の基準)	骨密度値**
正常	なし(骨萎縮なし)	YAMの80%以上
骨量減少	疑いあり(骨萎縮度I度)	YAMの70～80%
骨粗鬆症	あり(骨萎縮度II度以上)	YAMの70%未満

*YAM：若年成人平均値(20～44歳)
**骨塩定量の測定部位は，原則として閉経期以後(65歳未満)は腰椎，高齢者(65歳以上)は，大腿骨頸部とする.
(日本骨代謝学会，2000年度版一部改定)

表2　骨代謝マーカー

骨形成マーカー	
血清	骨型アルカリホスファターゼ(BAP)*
	オステオカルシン(OC)*
	I型コラーゲンN末端架橋テロペプチド(NTx)*
	I型プロコラーゲンC末端ペプチド(PICP)
	I型プロコラーゲンN末端ペプチド(NICP)
骨吸収マーカー	
血清	酒石酸抵抗性酸性ホスファターゼ(TRAP)
	I型コラーゲンC末端架橋テロペプチド(ICTP)
尿	ピリジノリン(PYD)
	デオキシピリジノリン(DPD)*
	I型コラーゲンC末端テロペプチド(CTx)
	I型コラーゲンN末端架橋テロペプチド(NTx)*

*保険収載検査(2004年10月現在)

b.　活性型 $VitD_3$ 製剤

活性型 $VitD_3$ 製剤には，骨密度維持効果が示されており，GC性骨粗鬆症の基礎薬物療法となる．高Ca血症，高Ca尿症に留意する.

c.　カルシトニン製剤

カルシトニン製剤は，骨吸収抑制作用に加えて，鎮痛作用を有する．腰背部痛を伴う高代謝回転型骨粗鬆症には第一選択となる.

d.　ビスホスホネート製剤

摂取後は直ちに骨中のハイドロキシアパタイトに吸着して，骨吸収を強力に抑制する．エチドロネートは大量投与により骨形成を抑制するため，200～400 mg/日を2週間投与，10～12週間休薬を1クールとする周期的間歇投与を行う．アレンドロネートは，骨の石灰化を障害せずに骨吸収を強力に抑制し，骨量増加と骨折予防効果を示す．5 mg/日を連日投与する．リセドロネートは連日2.5 mg/日を服用する．大腿骨頸部骨折予防効果が示されており，高齢者での効果が見込まれる．本剤は妊婦，高度腎障害患者への投与は禁忌である.

e.　その他の薬剤

ほかに，イプリフラボン製剤，エストロゲン製剤，SERMおよび $VitK_2$ 製剤などがある．SERMは，欧米での大規模試験で椎体骨折の高い予防効果や虚血性心疾患高リスク群で発症抑制効果が示されている.

おわりに

大規模臨床試験に基づいたGC性骨粗鬆症の治療および予防ガイドラインの作成が望まれる.

文　献

1) American College of Rheumatology Ad Hoc Committee on glucocorticoid-induced osteoporosis. Recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis：2001 update. Arthritis Rheum 44：1496-1503, 2001
2) Sato, S., Ohosone, Y., Suwa, A. et al.：Effect of intermittent cyclical etidronate therapy on corticosteroid induced osteoporosis in Japanese patients with connective tissue disease：3 year followup. J Rheumatology 30：2673-2679, 2003

総　説

ヒストン蛋白を標的とする自己抗体の特異性と臨床免疫学的意義

諏訪　昭

総　説

ヒストン蛋白を標的とする自己抗体の特異性と臨床免疫学的意義

諏訪　昭

Specificities and clinical significance of autoantibodies directed against histones

Akira SUWA

Department of Internal Medicine, Keio University School of Medicine

(Received April 4, 2005)

summary

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the occurrence of numerous autoantibodies directed against nuclear antigens. Anti-histone antibodies (AHA) are as prevalent as their anti-dsDNA counterparts in SLE. Despite their frequency and potential importance, there have not been given much attention to AHA until recently. Nucleosomes, the fundamental repeating units of the chromatin, are formed of complexes of histones and DNA. The nucleosome core particle is composed of a central tetramer of 2 molecules each of H3 and H4 flanked by 2 dimers of H2A and H2B and surrounded by 2 superhelical turns of approximately 146 base pairs of DNA. The full nucleosome conatins a molecule of H1 located at the point where DNA enters and exits the nucleosome. Recent studies have shown that the post transcriptional modification of histone changes chromatin structure to regulate transcription and the concept of this mechanism "epigenetics" has become center of attention in the field of basic cell biology.

There have been described diverging specificities of AHA. Many attempts to locate antigenic determinants recognized by AHA have been made and H1 and H2B have been thought as common targets in lupus patients. Studies on murine models of lupus have shown several intresting findings. The universal epitope is located on H2B in (NZBxNZW)F1 mice. In addition to core histones, MRL-MP/Fas^{lpr} mice develop high titers of autoantibodies to H1. Autoimmunity to chromatin regularly involves humoral immune responses directed against H1. These histones appear to be an early (possibly initial trigger) autoantigen for this autoimmune response in lupus.

Key words——autoantibody; epigenetics; histone; nucleosome; systemic lupus erythematosus

抄　録

ヒストンはクロマチンの最小基本単位であるヌクレオソームを構成する蛋白成分であり，コアヒストンH2A, H2B, H3, H4とリンカーヒストンH1の5種のサブユニットから構成される．近年ヒストンの構造と機能の解析が進み，エピジェネティクスがヒストン蛋白の翻訳後修飾によるクロマチンの構造変化によって制御されていることが明らかにされた．ヒストンは，二本鎖DNAとともにSLE患者における主要な自己抗原であるが，プロカインアミドやヒドララジンなどによる薬剤誘発性ループスにおいても抗ヒストン抗体産生を特徴とし，その病因的意義が注目されている．また，自然発症SLEモデルマウスにおいて，コアヒストン，リンカーヒストンに加えて，ヌクレオソームが主要なループス抗原であることや，H1やヌクレオソームに対する免疫応答が自己免疫のトリガーとなる可能性も示されている．ヒストンを標的とする自己抗体産生機序の追求は，SLEの病因，病態を解明する上で，重要と考えられる．

はじめに

膠原病は原因不明の炎症性疾患であり，自己細胞成分に対する多彩な自己抗体産生を特徴とする．これらの自己抗体は特定の臨床像と密接に関連し，診断や治療反応性，予後推定など臨床的に有用であるばかりでなく，細胞内分子の構造と機能解明にも役立つ[1～3]．全身性エリテマトーデス（SLE）患者血清中には，抗二本鎖DNA（dsDNA）抗体，抗ヒストン抗体，抗Sm抗体，抗リボソームP抗体など種々の自己抗体が高頻度に検出される[1～4]．抗ヒストン抗体は，当初SLE患者に発見され，その後薬剤誘発性ループス（drug-induced lupus erythematosus；DLE）患者で報告された[4～6]．ヒトおよび自然

慶應義塾大学医学部内科

発症SLEモデルマウスの解析から，ヒストンとともにヌクレオソームが主要なループス抗原であり，ヌクレオソームに対する免疫応答がその構成成分であるDNAとヒストンに対する自己抗体産生のトリガーとなる可能性が示されている[4,7]．また，遺伝子配列の変化をともなわない情報記憶と遺伝子発現を行うための機構であるエピジェネティクスは，ヒストン蛋白の翻訳後修飾によるクロマチンの構造変化によって制御されていることが明らかにされ，ヒストンの翻訳後修飾はポストゲノム研究として基礎生物学の分野で大いに注目されている[8]．本稿では，ヒストンに対する自己抗体の特異性と臨床免疫学的意義について解説する．

1. ヌクレオソームの構造と機能

ヒストンはクロマチンの最小基本単位であるヌクレオソームを構成する蛋白成分であり，塩基性アミノ酸（アルギニンおよびリジン）に富み，トリプトファンを含まない塩基性蛋白である．全ての有核細胞にはヒストンが存在し，そのアミノ酸配列は進化を通じて保存される．ヒストンは5種のサブユニット，H1（21 kDa），H2A（14.5 kDa），H2B（13.7 kDa），H3（15.3 kDa），H4（11.3 kDa）から構成される．H2A, H2B, H3, H4は各々2分子が結合したオクタマー（コアヒストン八量体）を形成し，その周囲にDNAが2回転（146bp）巻きついてヌクレオソームとなる（図1）[9]．H1はリンカーヒストンと呼ばれ，ヌクレオソーム構造には関与せず，ヌクレオソーム間の結合とクロマチン構造保持に関わるほかに，細胞分化との関連も指摘されている[9]．ヒストンには多くのバリアントがあるが，H1はさらに多くのサブタイプに分けられる．各サブタイプの発現は組織によって異なり，細胞分化の間や細胞周期によっても異なる．脊椎動物では，H1以外に卵母細胞特異的なB4，最終分化細胞特異的なH5，$H1^0$，精子特異的なH1tに大別される．H1バリアントは構造上C末端尾部のアミノ酸の数および組成が異なっており，発生後期のH1バリアントほどクロマチン結合能が高くなる[10]．

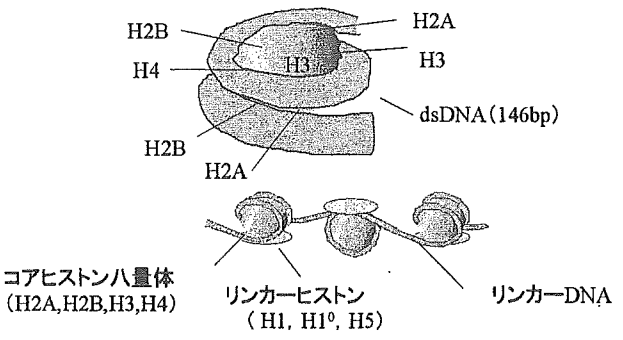


図1 ヌクレオソームの構造
H2A，H2B，H3，H4は各々2分子が結合したオクタマー（コアヒストン八量体）を形成し，その周囲にDNAが2回転（146bp）巻きついてヌクレオソームとなる．H1（リンカーヒストン）はヌクレオソーム間の結合とクロマチン構造保持に関わる．

ヌクレオソームが形成されるためには，ヒストン運搬蛋白であるヒストンシャペロンNAP-1（nucleosome assembly protein 1）やCAF-1（chromatin assembly factor-1）によるヒストン転移が必要である．すなわち，最初にH3, H4がDNA上に先行して転移され，つぎにH2A, H2Bが転移してコアヌクレオソームが形成される．この後，スペーシング因子ACF（ATP dependent chromatin assembly and remodeling factor）がATPのエネルギーを用いてヌクレオソームを可動化し，ヌクレオソームアレイを形成する．ヒストンは，アセチル化，リン酸化，メチル化，ADPリボシル化，モノユビキチン化などの翻訳後修飾によって，クロマチンの構造や活性を変化させ，転写，複製，修復など多様な生物学的機能を制御すると考えられている[8]．

2. ヒストン抗原の精製

ヒストン抗原は，ニワトリ有核赤血球，マウス肝臓などからHPLCにより精製される[11]．精製方法によって，ヒストン抗原の立体構造は影響を受けやすく，また，精製ヒストン抗原はリオフィライズ後に保存されるが，試料中に混入した水分により分解を受けやすいため，長期保存は適さない．水溶液に溶解後は，凝集して抗原性が変化しやすく，4℃で1-2週間以内に用いることが望ましい[12]．精製ヒストンの純度は，15-18%ドデシル硫酸ナトリウム-ポリアクリルアミドゲル電気泳動（SDS-PAGE）で確認される．ラット肝よりヒストンを精製した成績を示す（図2，図3）[13]．仔ウシ胸腺を初めとする異なる種，組織由来の精製ヒストンが市販されているが，純度や抗原性を確認の上，抗原として用いることが必要である．ヒストンをコードするcDNAを用いたリコンビナント蛋白を抗原とした抗体測定系は一般的でない．

3. 抗ヒストン抗体の特異性

抗ヒストン抗体は，当初SLE患者に発見され，

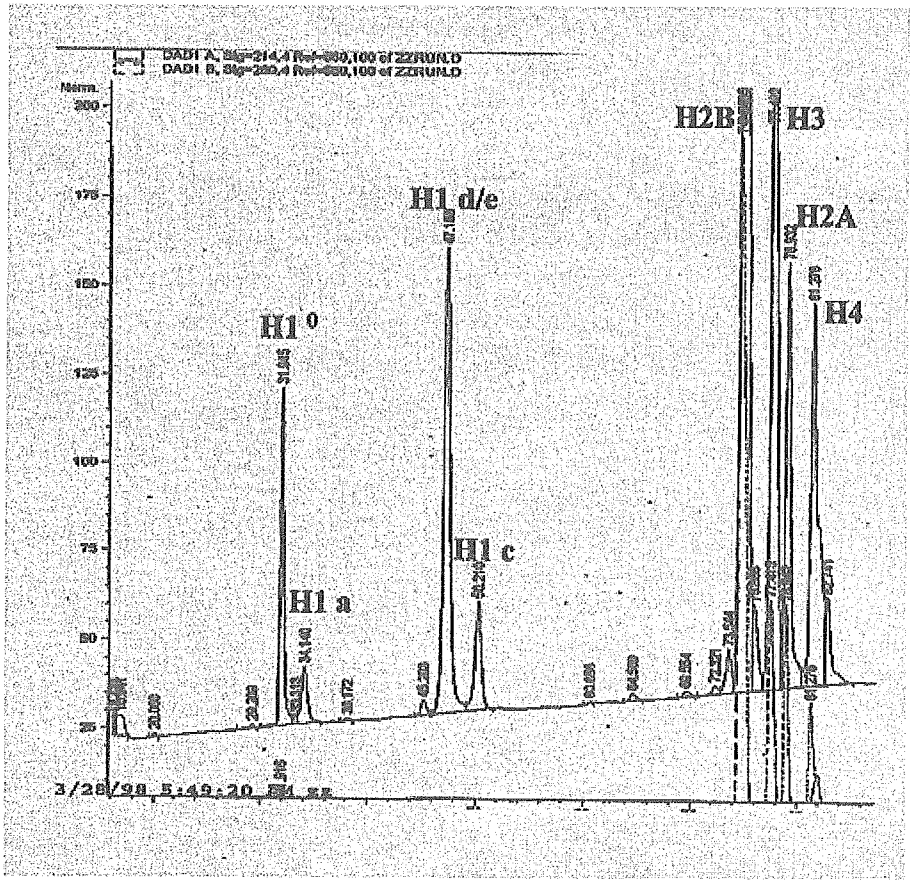


図2　FPLCを用いたヒストンの精製
FPLCを用いラット肝よりヒストンを精製した．(文献13)より引用)

その後DLE患者で報告された[4]．抗ヒストン抗体は抗dsDNA抗体と同様にSLEに高頻度に検出され，DLEでは抗ヒストン抗体測定が診断上有用である．しかしながら，抗ヒストン抗体は他の自己抗体に比して，注目を集めることは少なかった．その理由として，抗原の調整に手間がかかる上に，精製抗原が分解されやすいこと，抗体検出法により成績が異なり，解釈が分かれたことなどが，考えられる．抗ヒストン抗体はLE細胞形成に必要なLE因子そのものとする説がある．この説には異論もあるが，DLEではLE細胞陽性率が高い．抗ssDNA抗体は抗ヒストン抗体と併存しやすく，DLEでの陽性率が高い．

1. 抗ヒストン抗体測定法

抗ヒストン抗体測定法として，補体結合反応，蛍光抗体法[14]，RIA[15]，ELISA[16]，免疫ブロット法[4]などが開発されてきた．抗ヒストン抗体はHEp-2細胞を基質とする蛍光抗体法で核の均質型染色を示す．本法のみでは抗ヒストン抗体を同定できず，通常の核材，塩酸処理した核材，精製ヒストンを再構成させた核材を用いた三段階の蛍光抗体法[14]により抗ヒストン抗体を検出する．本法では抗H2A-H2B抗体以外の抗ヒストン抗体は陰性となることがあること，手技が煩雑であること，定量的でないことより現在は行われていない．

ELISAは精製ヒストン各亜分画が入手可能な場合に行われる．ELISAは高感度で，多数検体を処理できるという利点があるが，非特異的反応がみられることや，抗原の固相化により，confomationalエピトープの一部が認識されにくくなる可能性がある．抗ヒストン抗体のELISAキットも市販されている．

免疫ブロット法では，精製ヒストンを15-18%SDS-PAGEで分画後，ニトロセルロース膜に転写し，患者血清，ついで酵素標識抗ヒトIgGと反応させ，発色反応により抗体を検出する．本法は感度と特異性が高く，各亜分画に分離していない抗原を用いた場合でも各亜分画特異抗体を検出できる利点があるが，手技が複雑でスクリーニングには適さない．また変性ヒストン蛋白を抗原とするため，エピトープ反応性はELISAのそれと異なる．