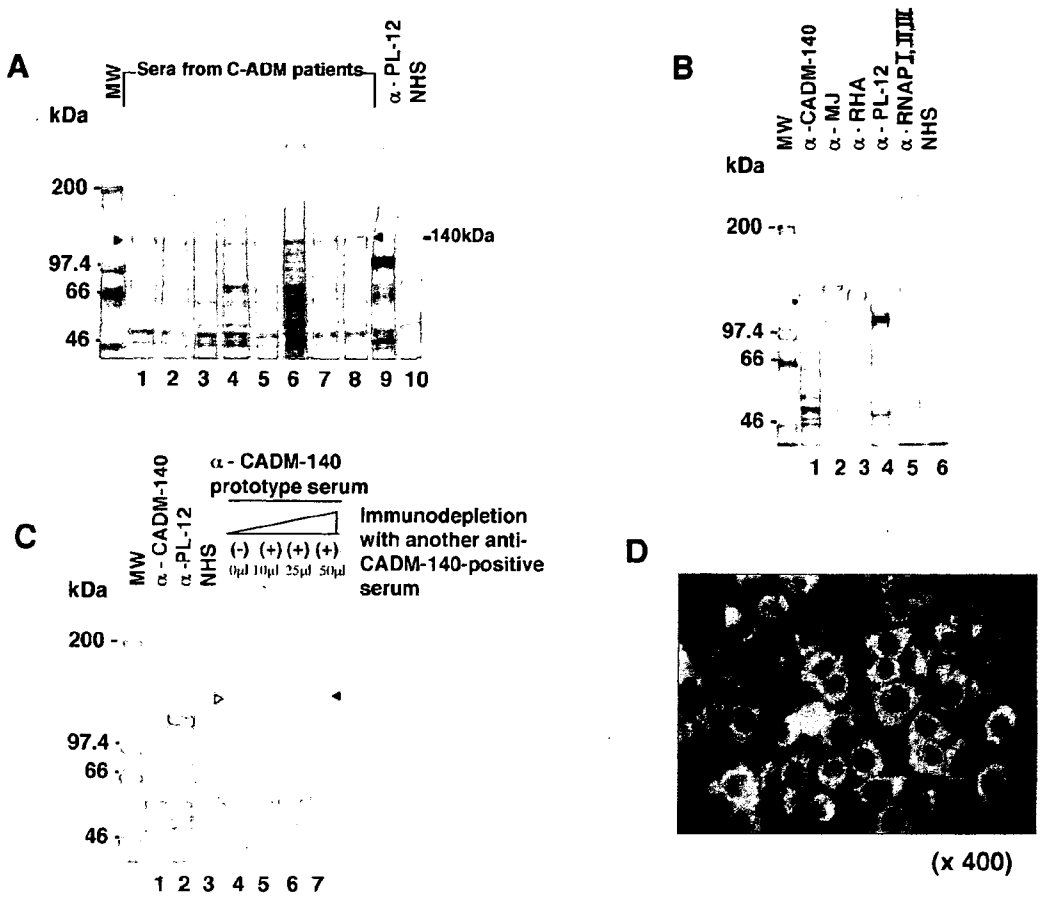

Figure 1. **A,** Immunoprecipitation of polypeptides with sera from patients with clinically amyopathic dermatomyositis (C-ADM), using ^{35}S-methionine–labeled K562 cell extracts. Lanes 1–8, Sera from C-ADM patients; lane 9, anti–PL-12 serum; lane 10, control normal human serum (NHS). A 140-kd protein 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.

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Clinical characteristics of Japanese patients with anti-PL-7 (anti-threonyl-tRNA synthetase) autoantibodies

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Abstract

Objective

The clinical and laboratory features of seven Japanese patients with anti-aminoacyl-tRNA synthetase (ARS) autoantibodies against PL-7 (anti-threonyl-tRNA synthetase) were analyzed and compared with previously published findings.

Methods

Serum samples from 1,135 Japanese patients with various autoimmune diseases were screened for anti-PL-7 antibodies using RNA and protein immunoprecipitation assays. The patients whose sera contained anti-PL-7 antibodies were assessed regarding clinical symptoms and clinical course.

Results

Sera from seven patients were found to have anti-PL-7 antibodies. These autoantibodies were associated with polymyositis/dermatomyositis (PM/DM) and/or interstitial lung disease (ILD). The clinical diagnoses of these seven patients were PM - systemic sclerosis (SSc) overlap (5 patients), DM (1 patient) and idiopathic pulmonary fibrosis (IPF) (1 patient). All patients had ILD with a chronic course and six also had arthritis (85%) and five sclerodactyly (71%).

Conclusions

These results indicate that anti-PL-7 autoantibodies are closely associated with PM-SSc overlap as well as ILD, arthritis and sclerodactyly in our series of Japanese patients.

Key words

Polymyositis/dermatomyositis (PM/DM), interstitial lung disease (ILD), anti-aminoacyl-tRNA synthetases (ARS) antibodies.

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Introduction

The aminoacyl-tRNA synthetases are a set of cellular enzymes, each of which catalyzes the formation of aminoacyl-tRNA from a specific amino acid and its cognate tRNA. Autoantibodies to six anti-aminoacyl-tRNA synthetases (anti-ARS) have been identified in patients with inflammatory myopathies, as follows: anti-histidyl (anti-Jo-1), anti-threonyl (anti-PL-7), anti-alanyl (anti-PL-12), anti-glycyl (anti-EJ), anti-isoleucyl (anti-OJ), and anti-asparaginyl (anti-KS) tRNA synthetases (1-10). Among these anti-ARS antibodies, the most common, anti-Jo-1, are found in approximately 20-30% of polymyositis/dermatomyositis (PM/ DM) patients (8, 10-11).

Each of these anti-ARS antibodies has been reported to be associated with a similar syndrome. This syndrome is characterized by myositis with a high frequency of interstitial lung disease (ILD) (50-80%) and arthritis (50-90%), as well as an increase (compared with the overall myositis population) of Raynaud's phenomenon (60%), fever with exacerbations (80%), and the skin lesions of the fingers referred to as "mechanic's hands" (70%) (1, 7). Although the similarity of clinical features in patients with different anti-ARS antibodies is striking, further observation and analysis has shown that there are certain differences in clinical symptoms associated with each of the anti-ARS antibodies.

Hirakata *et al.* examined clinical features of anti-synthetase syndromes in detail and reported that anti-Jo-1 antibodies are common in patients with myositis, but anti-PL-12 and anti-KS antibodies are found in patients with ILD without any signs of myositis (10). The latter are more likely to have ILD and/or arthritis without clinical evidence of myositis (10,12-13).

Anti-PL-7 antibodies are the first non-Jo-1 anti-ARS, found in patients with PM/DM accompanied by ILD, the frequency of which is low (2). In previous studies, this antibody was found in only 3-4% of all patients with PM/DM (2,6, 8,14). Targoff *et al.* reported that patients with anti-PL-7 antibodies had a high incidence of arthritis and ILD (15). However, the presence of anti-PL-7 antibodies and their clinical significance has not been reported in Japanese patients so far.

In the present study, we describe the clinical and laboratory features of Japanese patients with antibodies against anti-PL-7 and review previously published reports from elsewhere.

Patients, materials and methods

Patients and sera

Serum samples were obtained from 1,135 Japanese patients suspected of having connective tissue diseases seen at the current or previous collaborating centers of the authors between 1990 and 2000. These included 120 with PM/DM, 400 with systemic lupus erythematosus (SLE), 192 with systemic sclerosis (SSc), 58 with rheumatoid arthritis (RA), 101 with mixed connective tissue disease (MCTD)/overlap syndrome, 114 with idiopathic pulmonary fibrosis (IPF), and finally, 150 patients who had arthritis or erythema but did not meet the criteria for other connective tissue diseases.

PM/DM was diagnosed based on the criteria of Bohan and Peter (16). The assessment of muscle weakness was performed using a manual muscle test (17). The diagnosis of SSc was based on the criteria for the classification of SSc defined by the American College of Rheumatology in 1980 (18). ILD was considered to be present if an interstitial change was observed on both chest radiography and computed tomography (CT) or a restrictive pattern found on pulmonary function testing in patients with IPF or PM/DM.

Detection of anti-PL-7 antibodies

The immunoprecipitation (IPP) from HeLa cell extracts was performed as previously described (1, 6). For analysis of RNAs, 10 μl of patient sera was mixed with 2 mg of protein A-Sepharose CL-4B (Pharmacia Biotech AB, Uppsala, Sweden) in 500 μl of IPP buffer (10 mM Tris-HCl, pH 8.0, 500 mM NaCl, 0.1% Nonidet P-40) and incubated with end-over-end rotation (Labquake shaker; Lab Industries, Berkeley, CA) for 2 h at 4°C. The IgG-coated Sepharose was washed 4 times

in 500 μl of IPP buffer using 10-second spins in a microfuge and was resuspended in 400 μl of NET-2 buffer (50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.05% Nonidet P-40). This suspension was incubated with 100 μl of extracts, derived from $6x10^6$ cells, on the rotator for 2 h at 4°C. The antigen-bound Sepharose beads were then collected by centrifugation for 10 s in the microfuge, washed 4 times with NET-2 buffer, and resuspended in 300 μl of NET-2 buffer. To extract bound RNAs, 30 μl of 3.0 M sodium acetate, 30 μl of 10% SDS, 2 μl of carrier yeast tRNA (10 mg/ml; Sigma, St. Louis, MO) and 300 μl of phenol/chloroform/isoamyl alcohol (50: 50: 1, containing 0.1% 8-hydroxyquinoline) were added to the Sepharose beads. After agitation in a Vortex mixer and spinning for 1 min, RNAs were recovered in the aqueous phase after ethanol precipitation and dissolved in 20 μl of electrophoresis sample buffer composed of 10 M urea, 0.025% bromophenol blue, and 0.025% xylene cyanol-FF in TBE buffer (90 mM Tris-HCl, pH 8.6, 90 mM boric acid, and 1 mM EDTA). The RNA samples were denatured at 65°C for 5 min and then resolved in 7 M urea-10% polyacrylamide gels, which were then silver-stained (Bio-Rad Laboratories, Hercules, CA).

For the protein studies, antibody-coated Sepharose beads were mixed with 400 μl of [^{35}S] methionine-labeled HeLa extracts derived from $2x10^5$ cells, and rotated at 4°C for 2 h. After four washes with IPP buffer, the Sepharose beads were resuspended in SDS - sample buffer (2% SDS, 10% glycerol, 62.5 mM Tris-HCl, pH 6.8, 0.005% bromophenol blue). After heating (90° C for 5 min), the proteins were fractionated by 10% SDS-PAGE, enhanced with 0.5 M sodium salicylate, and dried. Radiolabeled protein components were analyzed by autoradiography.

With these assays, anti-ARS, anti-signal recognition particle, anti-Mi-2, anti-SSA, anti-SSB, anti-U1-RNP, anti-Scl-70, anti-PM-Scl and anti-Ku autoantibodies are detectable in comparison with corresponding standard sera (1). We also examined anticentromere antibody by ELISA (Medical & Biological Laboratories Co., Ltd. Nagoya, Japan).

Clinical features

Clinical information was retrospectively assessed in all PM/DM patients as well as non-PM/DM patients positive for anti-PL-7 antibodies. Clinical findings included clinical symptoms, serum creatine kinase (CK) level, electromyogram (EMG), muscle biopsy, chest radiograph and chest CT. The resolution of the myositis symptoms was defined as having both improvement of muscle weakness on a manual muscle test and the normalization of serum CK level. The two groups of PM/DM patients with or without anti-PL-7 antibodies were compared. Moreover, our cases were compared with those previously reported in the literature.

Statistical analysis

All comparisons between the two patient groups were performed using the χ^2 test. Significance level was set at 5%.

Results

Identification of anti-PL-7 antibodies

Of the 1,135 sera tested, samples from seven patients were found to immunoprecipitate a characteristic identical pattern of tRNAs. Representative examples are shown in Figure 1. This pattern of tRNAs was clearly distinguishable from those precipitated by the five other described anti-synthetases and identical in mobility and appearance to anti-PL-7 standard serum (Fig. 1a). The same sera also immunoprecipitated a protein band from [^{35}S] methionine-labeled HeLa cell extracts migrating at 80 kDa. This was clearly different from those immunoprecipitated by sera reactive with the other described anti-synthetases (Fig. 1b). Thus, it is concluded that they contained anti-PL-7 antibodies.

Clinical features of patients with anti-PL-7 antibodies

Clinical features of the 7 patients with anti-PL-7 antibodies are summarized in Table I. Five patients were clinically diagnosed as having PM-SSc overlap syndrome and the other two as DM and IPF. Six (86%) had muscle weakness and arthritis. Four (57%) had Raynaud's phenomenon. It was of note that 5 patients had scleroderma: the extent of skin thickness was diffuse scleroderma in 2 (29%), proximal scleroderma in one (14%) and sclerodactyly alone in 2 (29%). Although two (29%) had mechanic's hands, sclerodactyly of these patients was clearly distinguished from mechanic's hands. All 7 patients were diagnosed as having ILD from the results of chest radiography and chest CT or pulmonary function testing. One patient had anti-SS-A antibodies and another had anticentromere antibodies.

Characteristics of myositis in patients with anti PL-7 antibodies

The characteristics of 6 patients suffering from myositis are summarized in Table II. Only one patient manifested a DM rash and was accordingly diagnosed as having DM. The maximum level of CK (IU/l) was relatively low throughout the clinical course (maximum CK was 2,830 IU/l, seen in patient #1). EMG was performed in all 6 myositis patients and all showed a myogenic pattern: low-amplitude polyphasic units of short duration and resting fibrillation, complex repetitive discharges and positive sharp waves in needle EMG. The muscle biopsy revealed atrophic fibers, active necrosis with regeneration and infiltration of lymphocytes in all 3 patients tested. The administration of prednisolone (PSL) alone without other immunosuppressant in 5 resulted in an improvement of both muscle strength and serum CK value in all. One patient had no PSL medication due to concomitant tuberculosis infection. PSL was tapered gradually and 3 patients maintained inactive myositis by continuing on a low dose of PSL. Two patients (#1 and #3) died of cardiac failure and respiratory failure due to bacterial infection. The duration of the disease was 159 months and 44 months in these latter patients. All 7 PM/DM patients had ILD, classified as chronic course. The symptoms of ILD preceded muscle involvement in 5 patients.

Frequencies of several clinical mani-

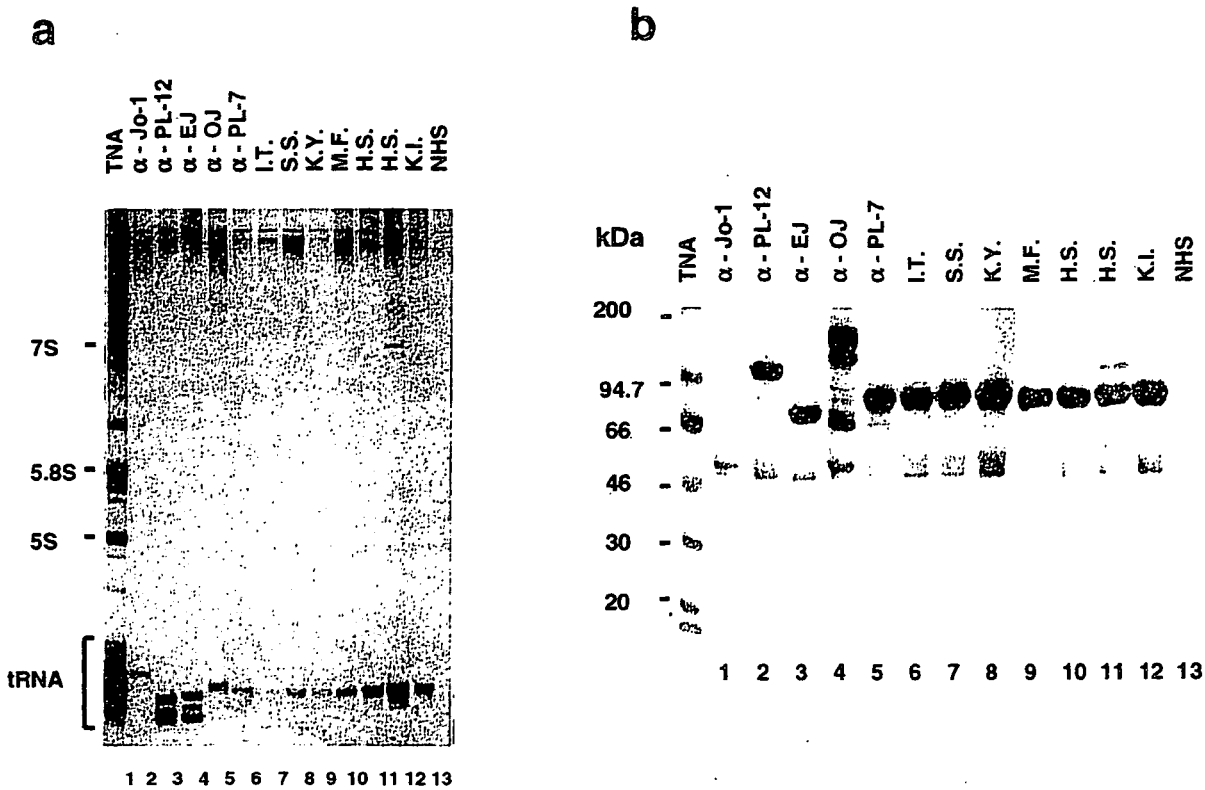

Fig. 1. (a) Immunoprecipitation (IPP) of nucleic acids with anti-PL-7 sera and controls. Urea (7 M) and 10% PAGE of phenol-extracted immunoprecipitates from HeLa cell extracts were developed with silver stain. TNA, total nucleic acids, with the 5.8 and 5.0 S small ribosomal RNAs and the tRNA region indicated. Sera used for IPP include: lanes 1-5, the anti-synthetase sera indicated, with antibodies to Jo-1 (histidyl-tRNA synthetase), PL-12 (alanyl-tRNA synthetase), EJ (glycyl-tRNA synthetase), OJ (isoleucyl-tRNA synthetase), PL-7 (threonyl-tRNA synthetase); lanes 6-12, anti-PL-7 sera as indicated; and lane 13, control serum (NHS, normal human serum). The tRNA pattern with anti-PL-7 sera is easily distinguishable from that of the other anti-synthetases.
(b) IPP of proteins with anti-PL-7 sera and controls. Autoradiogram of 10% SDS-PAGE of immunoprecipitates from [^{35}S] methionine-labeled HeLa cell extracts. Mr, molecular weight markers of the sizes indicated to the left (kDa). The sera used for IPP are the same as those in (a). The same characteristic pattern of 80 kDa protein bands was seen with each of the seven anti-PL-7 sera. The pattern was clearly different from the bands immunoprecipitated by sera against the other anti-synthetases.

Table I. Clinical features of patients with anti-PL-7 antibodies.

Clinical findings	#1	#2	#3	#4	#5	#6	#7
Age/ gender	51/ male	59/ female	32/ female	53/ female	51/ female	64/ female	57/ female
Diagnosis	DM	PM-SSc	PM-SSc	PM-SSc	PM-SSc	IPF	PM-SSc
Fever	(-)	(-)	(+)	(+)	(+)	(+)	(-)
Arthritis	(+)	(+)	(+)	(+)	(+)	(-)	(+)
Muscle weakness	(+)	(+)	(+)	(+)	(+)	(-)	(+)
Raynaud's phenomenon	(-)	(+)	(+)	(+)	(-)	(-)	(+)
Extent of scleroderma	None	Proximal scleroderma	Sclerodactyly alone	Diffuse scleroderma	Diffuse scleroderma	None	Sclerodactyly alone
Digital pitting scar	(-)	(+)	(+)	(+)	(-)	(-)	(+)
Mechanic's hands	(-)	(-)	(-)	(+)	(+)	(-)	(-)
ILD	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Hypergammagloblinemia	(+)	(+)	(-)	(+)	(+)	(-)	(-)
Sjögren's syndrome	(+)	(+)	(+)	(+)	(-)	(-)	(-)
Other autoantibodies	(-)	(-)	Anti-SSA	(-)	(-)	(-)	Anti centromere

PM: polymyositis; DM: dermatomyositis; SSc: systemic sclerosis; ILD: interstitial lung disease.

Table II. Characteristics of myositis in patients with anti-PL-7 antibodies.

Clinical and laboratory findings	#1	#2	#3	#4	#5	#7
DM rash	(+)	(-)	(-)	(-)	(-)	(-)
Maximun CK level (IU/l)	2,830	748	930	1,682	1,663	1,005
EMG findings	Myogenic*	Myogenic	Myogenic	Myogenic	Myogenic	Myogenic
Muscle biopsy	Myopathy	n.d.	n.d.	n.d.	Myopathy	Myopathy
Atrophy	(+)				(+)	(+)
Necrosis with regeneration	(+)				(+)	(+)
Infiltration of lymphocytes	(+)				(+)	(+)
Initial dose of PSL (mg/ day)	60	(-)	40	40	50	30
Duration of treatment (mos.)	159	(-)	44	24	110	93
Efficacy of PSL for myositis	(+)	n.d	(+)	(+)	(+)	(+)
Present status	Death	Alive	Death	Alive	Alive	Alive

*Low amplitude, resting fibrillation, positive sharpe wave (denervatrion potencials) were present. DM: dermatomyositis, CK: creatine kinase, EMG: electromyogram, PSL: prednisolone.

festations were compared between anti-PL-7-positive and negative PM/DM patients (Table III). The frequencies of ILD and sclerodactyly were found to be significantly higher in antibody-positive patients.

Comparison of the clinical features of patients with anti-PL-7 antibodies in the present study and those in the literature

The clinical features of patients with anti-PL-7 antibodies reported in the English-language literature were reviewed (2, 13, 15, 16, 19, 20). A summary of clinical data including our study is shown in Table IV. The frequencies of arthritis, myositis, and Raynaud's phenomenon in our series is similar to those of previously reported patients with anti-PL-7 antibodies. On the other hand, the occurrence of sclerodactyly in our series is greater compared with previous reports from North America and the United Kingdom.

Case 1 (patient #5)

This 51-year-old woman noticed dyspnea on exertion in 1995, after which symptoms progressively worsened. Her general practitioner identified an abnormal lung shadow in the chest radiogram. She was admitted to the Keio University Hospital in October 1995. She had dyspnea on exertion, and muscle weakness predominantly in the proximal muscle. She also had diffuse scleroderma and Raynaud's phenomenon. The CK level was elevated (1,663 IU/l). Myopathic changes detected by EMG mainly in proximal muscles and active necrosis with regeneration seen in a muscle biopsy specimen suggested the presence of myopathy. %VC was 59% and %DLco was 43% on lung function testing, indicating restricted respiratory impairment. A chest radiograph showed bilateral reticular shadow and infiltration. The chest CT revealed interstitial fibrosis and infiltration accompanied by air-bronchogram. A diagnosis of PM/SSc overlap syndrome was established based on proximal muscle weakness, elevated muscle enzymes, typical EMG and muscle biopsy findings and diffuse scleroderma. Treatment with 50 mg/day of PSL was started, resulting in improvement of clinical symptoms including muscle weakness, and dyspnea on exertion, and decrease in CK levels. However, dyspnea worsened again when the dose of PSL was tapered to 11 mg/day. In October 1997, she was re-admitted to our hospital and the dose of PSL was increased to 40 mg/day. %VC improved from the baseline (60%) to the level after treatment (74%). PSL was gradually tapered and she is now taking 10 mg/day of PSL. Although moderate dyspnea on exertion has persisted, she has no muscle weakness and serum CK level is within the normal range.

Case 2 (patient #7)

A 57-year-old woman developed dyspnea on exertion and had a non-productive cough in 1994. She was admitted to the Keio University Hospital in November 1994 due to worsening dyspnea. Chest radiography revealed a reticular shadow in both lower lung fields. A chest CT also showed bibasilar interstitial fibrosis. The pulmonary function test showed a decreased %VC (59%) and decreased $\%DL_{CO}$ (35%). A diagnosis of ILD was made, and PSL 40 mg/day was initiated, resulting in improvement of respiratory symptoms. The dose of PSL was tapered and discontinued in November 1995.

In August 1997, she gradually devel-

Table III. Comparison of clinical features in anti-PL-7-positive versus negative PM/DM.

Clinical and laboratory findings	Anti-PL-7(+) (n = 6)	Anti-PL-7(-) (n = 119)	P value
Male / female	1/ 5	36/83	NS
Fever (%)	3 (50)	59 (50)	NS
Arthritis (%)	6 (100)	73 (61)	NS
ILD (%)	6 (100)	52 (44)	P < 0.05
Raynaud's phenomenon (%)	4 (67)	35 (29)	NS
Sclerodactyly (%)	5 (83)	17 (14)	P < 0.005

* PM/DM: polymyositis/dermatomyositis, ILD: interstitial lung disease.

Table IV. Clinical features of patients with anti-PL-7 antibodies in literature and our study.

	Previous reports from North America and the United Kingdom							Present study	P value
Year/ Author	1984 Mathews	1988 Targoff	1990 Marguerie	1994 Satoh	1995 Mchugh	1999 Wasko	Total	Sato	
No.	5	4	4	1	1	1	16	7	
Male: female	1:4	2:2	1:3	0:1	0:1	1:0	5:11	1:6	n.s.
Arthritis no. (%)	3 (60%)	4 (100%)	4 (100%)	1 (100%)	0	1 (100%)	13 (81)	6 (86)	n.s.
Myositis no. (%)	4 (80%)	4 (100%)	3 (75%)	1 (100%)	0	1 (100%)	13 (81)	6 (86)	n.s.
ILD no. (%)	1 (20%)	3 (75%)	3 (75%)	0	1 (100%)	0	8 (50)	7 (100)	n.s.
RP no. (%)	2 (40%)	1 (25%)	4 (100%)	0	1 (100%)	1 (100%)	9 (56)	4 (57)	n.s.
Sclerodactyly no. (%)	0	0	2 (50%)	0	1 (100%)	0	3 (19)	5 (71)	P < 0.05

ILD: interstitial lung disease, RP: Raynaud's phenomenon

oped muscle weakness and polyarthralgia. In January 1998, the patient was re-admitted. She had sclerodactyly and digital pitting scar as well as muscle weakness and polyarthralgia. Blood tests revealed an elevated CK level (1005 IU/l). The EMG showed myopathic changes. A muscle biopsy revealed chronic inflammatory cell infiltrates in the endomysium, indicating myopathy. The diagnosis of PM-SSc overlap syndrome was made and administration of PSL 30 mg/day was reinstated. The muscle weakness and arthralgia were improved markedly and the CK level normalized in 1998.

Discussion

In the present study, we found 7 patients who had anti-PL-7 autoantibodies among 1,135 patients suspected to have CTD. With regard to clinical symptoms, the features of these patients with anti-PL-7 appeared to reside within the spectrum of the "anti-synthetase syndrome" that has been noted in other patients with anti-ARS antibodies (13). However, it should be noted that the frequency of sclerodactyly in our series was significantly higher than in our PM/DM patients without anti-PL-7 antibodies or anti-PL-7 antibody-positive patients previously reported in the English-language literature. In addition, 2 patients had diffuse scleroderma and one had proximal scleroderma. In fact, 5 of 7 (71%) patients were diagnosed as having PM-SSc overlap syndrome. Anti-PL-7 antibodies are likely to be associated with PM-SSc overlap syndrome in Japanese patients. It is thought that there could be certain racial difference in frequencies of autoantibodies. For instance, anti-PM-Scl antibodies known to be associated with PM-SSc overlap were detected in Caucasian SSc patients but not in Japanese SSc patients (21). Because the number of patients with anti-PL-7 is limited, further studies are required to confirm our hypothesis.

Refractory myositis with anti-ARS antibodies has been reported (22). However the degree of myositis of our cases was relatively mild. Treatment with corticosteroid alone resulted in the resolution of muscle weakness and the normalization of serum CK level successfully in all patients although 2 died from complications unrelated to myositis.

Arthritis and chronic ILD are characteristics of anti-ARS seropositive patients (7, 8) and these features were frequently detected in our series of patients with anti-PL-7 antibodies. It is known that certain patients with PM/DM have ILD preceding the appearance of muscle symptoms (1,8,23). Although patient #6 was diagnosed with IPF at this point, the possibility remains that muscle symptoms may arise in the future. Therefore, continuous careful follow-up observation will be necessary to monitor future muscle involvement.

In conclusion, clinical features detected in 7 Japanese patients with anti-PL-7 antibodies are essentially consistent with anti-ARS syndrome previously reported, such as high frequencies of arthritis, chronic ILD and relatively mild PM/DM for which corticosteroid therapy is effective. An additional clinical manifestation unique to anti-PL-7-positive patients is concomitant scleroderma, and anti-PL-7 are likely to be associated with PM-SSc overlap syndrome in Japanese patients. The detection of anti-PL-7 antibodies may be useful in the diagnosis and disease classification of patients with connective tissue diseases.

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

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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.

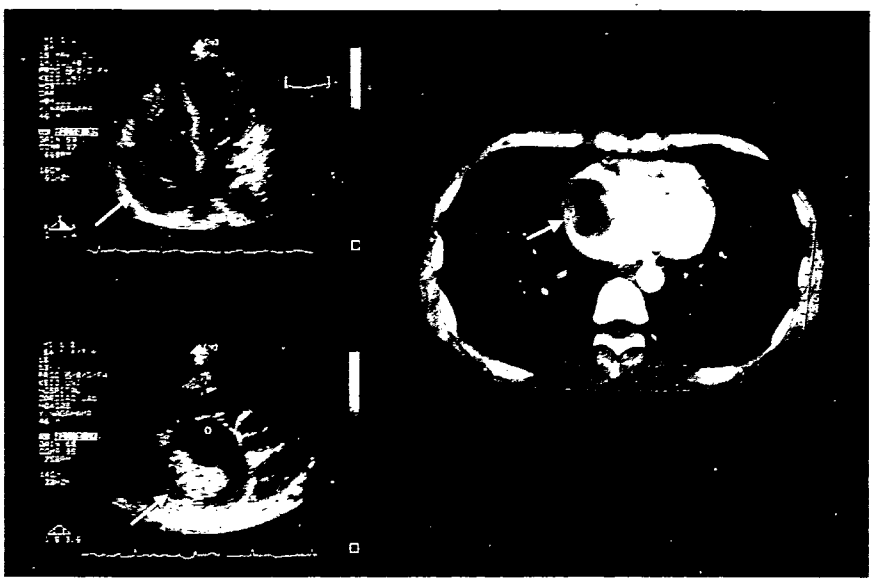
Case report

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.

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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

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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

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- 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．

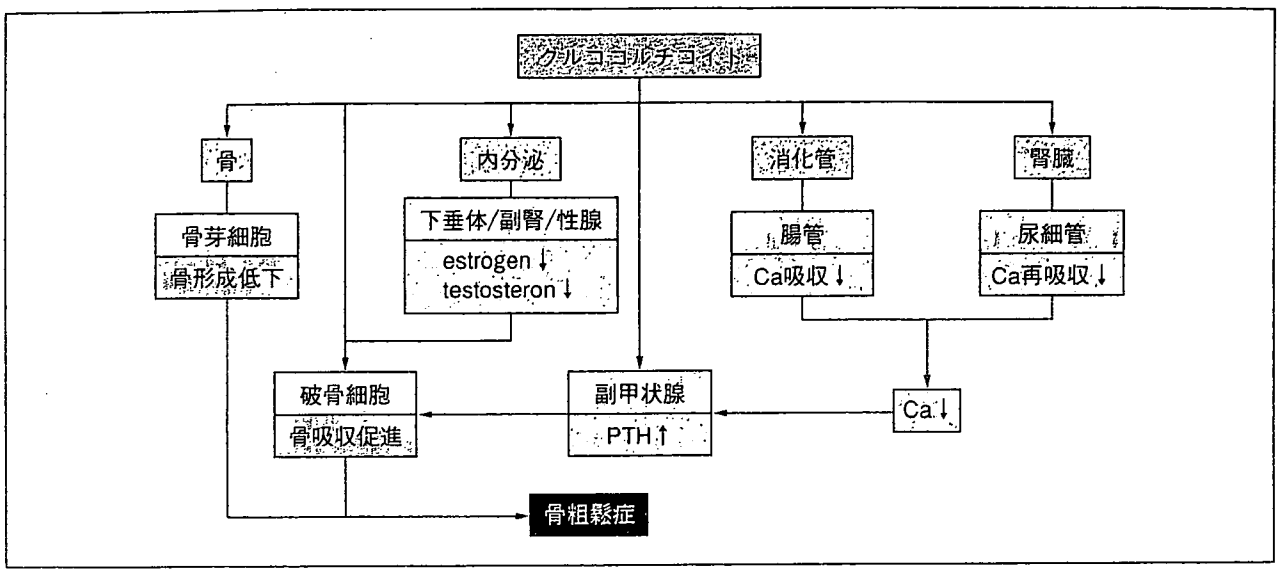

図1　GC 性骨粗鬆症の病態

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

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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

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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]．

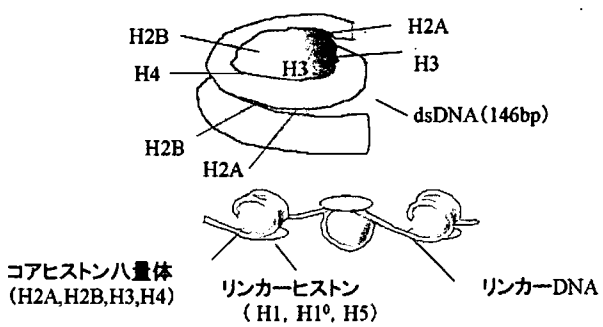

図 1 ヌクレオソームの構造
H2A，H2B，H3，H4 は各々 2 分子が結合したオクタマー（コアヒストン八量体）を形成し，その周囲に DNA が 2 回転 (146bp) 巻きついてヌクレオソームとなる．H1（リンカーヒストン）はヌクレオソーム間の結合とクロマチン構造保持に関わる．

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，

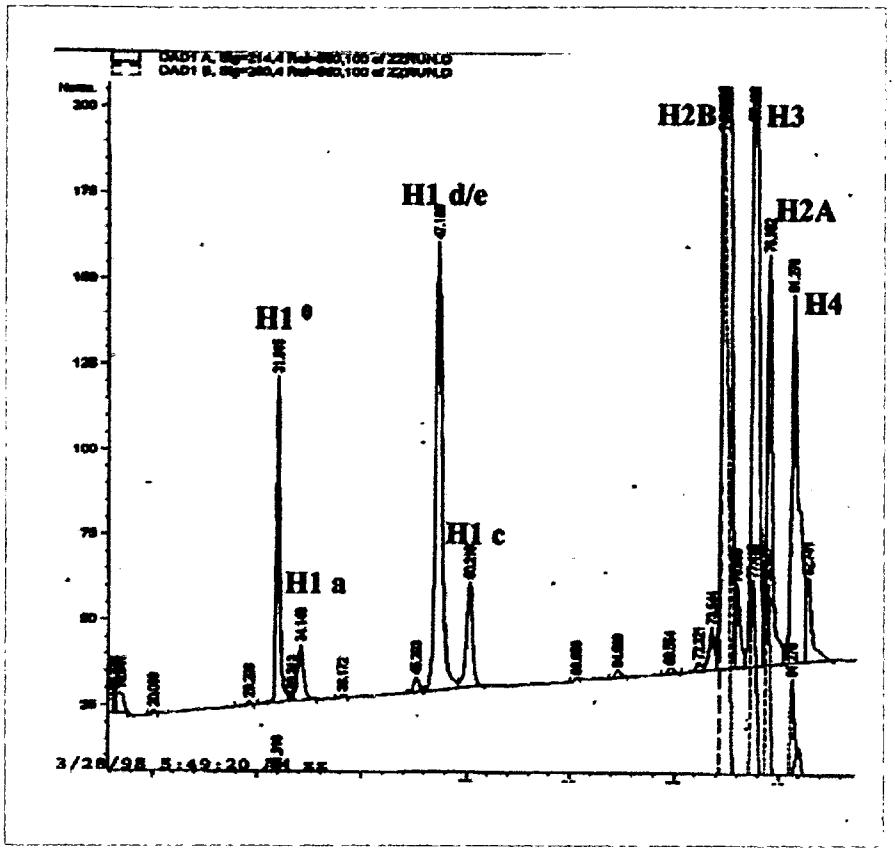

図2 FPLCを用いたヒストンの精製
FPLCを用いラット肝よりヒストンを精製した.(文献13)より引用)

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.