多発性筋炎に無症候性原発性胆汁性肝硬変症を 合併し好酸球増多を示した1例

武井 正美・山上 敬司・西成田 進・三田村 巧
池田 悦子・荒川 泰行*・田中 直英*・天木 秀一*
澤田 滋正・堀江 孝至

Jpn. J. Clin. Immun., 14 (1) : 98~104, 1991.

Polymyositis associated with asymptomatic primary biliary cirrhosis and eosinophilia : An analysis of the mitochondrial antigens by Western blotting

Masami Takei, Keiji Yamagami, Susumu Nishinarita, Ko Mitamura,
Etsuko Ikeda, Yasuyuki Arakawa*, Naohide Tanaka*, Shuichi Amaki*,
Shigemasa Sawada and Takashi Horie

*The First Department and *The Third Department of Internal Medicine,
Nihon University School of Medicine*

Key words : polymyositis,
primary biliary cirrhosis,
mitochondrial antigen,
Western blotting

【概 要】

多発性筋炎にて入院，胆道系酵素の上昇を認め，抗ミトコンドリア抗体（AMA）の高値，腹腔鏡下肝生検にて無症候性原発性胆汁性肝硬変症（APBC）を強く疑わせ，経過中好酸球の増多を示した1例を経験したので報告する。症例は44歳女性で昭和63年9月ころより筋の脱力感，圧痛，発熱を認め近医にて入院していたが，軽快せず，当院紹介入院となった。当院にて筋原性酵素の上昇，筋電図にて筋原性の異常，筋硬結部の筋生検にて間質へのリンパ球浸潤，筋繊維の大小不同，壊死，核の中心化，脂肪組織の浸潤を認め，臨床症状とも合わせ多発性筋炎と診断した。入院経過中，胆道系酵素の上昇を認め，AMAも高値を示したため，腹腔鏡下肝生検を施行，早期の無症候性原発性肝硬変を強く疑わせる所見を得た。さらには，AMAの亜分画をミトコンドリアM2分画を抗原としたウェスタンブロット法にて測定したところ，70 kDに特異的に結合するAMAの存在を認めた。経過中，好酸球増多を持続的に認めたが，IgEは正常で，臨床症状も伴わず，肝，筋への浸潤も認められないことから好酸性多発性筋炎は否定した。知りうるかぎりでは，多発性筋炎とAPBCの合併は10例前後の報告がみられるが，多発性筋炎が先行したものはほとんど報告がなく，AMAのウェスタンブロット法にて亜分

画の測定まで行ったものは見当たらないため、ここに報告する。

I. Summary and introduction

The association of anti-mitochondrial antibodies (AMAs) with primary biliary cirrhosis (PBC) is well known. Recent studies have revealed heterogeneity among AMAs with regard to the antigenic substrate. The substrates include components of the pyruvate dehydrogenase complex, including an E2 acetyltransferase with a relative molecular mass of ~ 70 kD¹⁾. This acetyltransferase is the 70 kD component of the group of mitochondrial antigens designated "M2". Autoantibodies to this substrate are highly specific for PBC. We here report a case of polymyositis (PM) that was complicated by an early stage PBC. Serum from this patient contained AMA reactive with the 70 kD component of the M2 fraction isolated from mitochondria. In this case, the Western blotting technique proved to be a very useful tool in supporting the diagnosis of PBC.

II. Materials and methods

PBC sera : Sera containing AMAs were obtained from patients with PBC in our out patient clinic. The diagnosis of PBC was based upon an evaluation of the patient's clinical symptoms, laboratory data, and the presence of AMA as determined by immunofluorescence²⁾.

Isolation of mitochondria : Mitochondria were isolated from calf heart using standard methods³⁾.

Western blotting : SDS gel electrophoresis was performed as described by Laemmli⁴⁾, and the mitochondrial proteins that resulted were transferred to a nitrocellulose membrane⁵⁾. Following that, immunoblotting and enzyme linked immunostaining were performed as described by Hadikusumo et al.⁶⁾.

III. Case report

A 44 year old female was first seen at a private out patient clinic in September, 1989. She exhibited muscle weakness which was most noticeable on climbing stairs and on holding her laundry. She had a fever of unknown origin and manifested

Raynaud's symptom and dysphagia. She had no past history or family history of any autoimmune diseases. On her subsequent referral to our clinic in early October, 1988, her physical examination revealed muscle tenderness.

Her laboratory tests included normal levels of the serum alkaline phosphatase, the serum bilirubin and the serum cholesterol. However, the serum aldolase, the serum creatine kinase (CK), the serum lactic dehydrogenase (LDH) and the serum glutamic oxaloacetic transaminase (GOT) were found to be increased (Table 1). She was admitted to our hospital, Nihon University Hospital at Itabashi in Tokyo, in the middle of October, 1988.

The patient complained of muscle tenderness in her extremities. On physical examination, induration of the muscle of the lower distal extremities was noted. This induration took the form of "bowl muscle".

She had obvious proximal muscle weakness and atrophy of pelvic girdle muscles. Her laboratory tests were similar to those obtained at the clinic. Negative test included anti DNA antibody, anti Jo-1 antibody, anti RNP, anti Sm, anti Ro (SS-A), anti La (SS-B), anti Scl-70, antibody to smooth muscle, and LE cell preparation.

However, tests for anti-nuclear antibody and rheumatoid factor were positive. Eosinophilia had been noted from the onset of her illness and was $1,190/\text{mm}^3$. Electromyography was consistent with a bilateral myogenic disorder in the lateral biceps brachii and the tibialis anterior and in the right brachioradialis. A biopsy of the induration of the muscle of the lower distal extremity revealed focal lymphocyte infiltration around the small vessels, single fiber necrosis of muscle cells and fibrosis in part within the muscle tissue (Figure 1). The results of this biopsy were considered to be consistent with polymyositis. Her pulmonary function and chest X-ray study showed no evidence of interstitial pneumonia or pulmonary fibrosis.

In November 1988, levels of serum IgM, IgG and IgA were elevated. Serum bile duct enzymes in-

Table 1 Laboratory data on admission

ESR 39 mm/h	Al-P 182 IU/l
WBC 7,000/mm ³	LAP 73 IU/l
Band 14%	γ -GTP 47 IU/l
Seg 17%	CK 1,695 IU/l (MM89%, MB11%)
Eo 17%	Aldolase 27 IU/l
Baso 1%	Myoglobin 650 mg/ml
Mono 10%	RA 2+
Lym 24%	RAHA 20x
Urinalysis	IgG 1,797 mg/dl
sug (-)	IgA 410 mg/dl
prot (-)	IgM 448 mg/dl
occult bleeding (-)	IgD 3.8 mg/dl
sediment n.p.	IgE 12 IU/dl
T. Prot 7.4 g/dl	ANA 3+320x
alb 52.7%	speckled
γ -gl 23.2%	Anti-DNA antibody (-)
TTT 13.1 KU	Anti-ENA antibody (-)
ZnTT 19.5 KU	Serological test for syphilis (-)
GOT 70 IU/l	Anti centromere antibody positive ;
GPT 42 IU/l	320 dilution (WNL < 20 dilution)
LDH 950 IU/l	LE cell (-)
Urine creatin-creatinine	HLA DR2
clearance 32%	Toxoplasma antibody 80 dils
	Pulmonary function (W. N. L.)
	V25 1.41/sec
	% VC 106%
	FEV _{1.0%} 88.1%

Figure 1 This section shows pieces of the skeletal muscle with focal inflammatory cell infiltration, mainly lymphocytes, particularly around small blood vessels in the muscle tissue. Some muscle fibers were atrophic. Fibrinoid angitis is not obvious.

Figure 2 Western blot analysis of AMAs with a mitochondrial M2 fractions. Lane 1 shows molecular weight marker protein, 74 kD phosphorylase b, 67 kD fetal bovine albumin, 45 kD ovalbumin, and 30 kD carbonic anhydrase. Lane 2 and 6 show normal control sera. Lane 3 and 7 show PBS sera containing high titer AMA detected immunofluorescence. Lane 4 and 5 show the patient sera obtained at different date.

Figure 3 The section shows a regular arrangement of hepatic cell cords. The portal areas are slightly expanded and contain mononuclear inflammatory cells, mainly lymphocytes and plasma cells. Some portal triads show granulomatous changes, associated with destruction or proliferation of bile ductules.

creased, and her AMA titer was positive (1 : 160). Smooth muscle antibodies were not present. Western blot analysis of her AMA, using the M 2 fraction from the mitochondria, revealed that her serum AMA bound specifically to the 70 kD polypeptide of

the M 2 fraction (Figure 2). A biopsy of her liver revealed early stage PBC (Figure 3).

Thus, a diagnosis of polymyositis complicated with stage 1 PBC was made, and a therapy of prednisolone (60 mg/day) and ursodeoxycholic acid

(30 mg/day) was commenced. Muscle power rapidly improved and the CK and other muscle enzymes levels reverted to a normal range. The IgM level also decreased but more slowly than the other immunoglobulins. Her serum bile duct enzyme levels also reverted to a normal range three weeks after treatment with prednisolone and ursodeoxycholic acid. But we did not try a liver biopsy after these therapy. Induration of the muscle of the lower distal extremities did not remarkably changed two months after treatment with prednisolone. An upper gastrointestinal examination, a barium enema, an ultrasonic examination, a whole-body computer tomography examination, and a gynecological examination all revealed normal findings. There was no clinical evidences of systemic lupus erythematosus, progressive systemic sclerosis, CREST syndrome, polyarteritis nodosa, Sjögren's syndrome, rheumatoid arthritis, or Churg-Strauss syndrome.

IV. Discussion

PM or PBC can be linked with other autoimmune or systemic diseases⁷⁻⁹⁾, and the association of PBC with Sjögren's syndrome⁸⁾, rheumatoid arthritis⁷⁾, and scleroderma⁹⁾ is well-known. PM, however, has not been frequently associated with PBC and, to our knowledge, less than 10 cases have been reported^{10,11)}. In most of these cases, PBC was followed by PM. All of these cases had positive AMA titers. Recently, several investigators have uncovered a heterogeneity in AMAs, and this has led to their classification into seven subclasses, designated M 1 to M 7¹²⁾. Antibodies against M 2 and M 4 in particular have been detected in serum from patients with PBC or related diseases. Most recently, it has been reported that AMAs directed against the trypsin sensitive (M 2) antigens of the inner mitochondrial membrane were specifically found in sera from patients with PBC^{13,14)}.

AMAs are clearly associated with PBC, but as AMAs can be detected in sera from other diseases, they are not completely specific for PBC. The specificity is increased by specifically determining anti-M 2 antibodies. Furthermore anti-M 2 anti-

bodies in sera from patients with PBC have reacted with 8 proteins in calf mitochondrial molecular masses of 72, 67, 59, 57, 56, 52, 45, 42 kD. Proteins of 72 and/or 67 kD have been commonly detected in all sera containing the anti-M 2 antibodies¹⁵⁾, and anti-M 2 antibodies have reacted with the major 70 kD and 52 kD antigens when tested against human placental mitochondria¹¹⁾. This 70 kD antigens, reacted by autoantibodies in patients with PBC is E 2 acetyltransferase of the pyruvate dehydrogenase complex.

Berg et al. have demonstrated that AMA in sera from patients with chronic active hepatitis reacts with the M 4 fraction of mitochondria, obtained by treating the M 2 fraction with trypsin. The anti-M 4 antibodies generally bind to a single 39 kD polypeptide, a substrate that completely differs from that of the polypeptides in the M 2 fraction¹³⁾. In our case, the patient's AMAs specifically reacted with the 70 kD M 2 polypeptide, as determined by Western blotting analysis. Thus, Western blotting analysis can be a useful and important diagnostic tool to distinguish the substrate specificities of AMA.

Okano et al. demonstrated that the co-existence of anti centromere antibodies with anti mitochondrial antibodies was significantly more frequent than with other defined autoantibodies¹⁶⁾. The existence of anti centromere antibodies with anti mitochondrial antibodies in our patient supports their observation.

It was also of interest that our patient had eosinophilia. The most common cause for mild eosinophilia in hospitalized patients is probably some from drug allergy, but eosinophilia was present in our patient prior to hospitalization. Parasitic infections cause eosinophilia, especially during the invasive phase. Also eosinophilia is usually mild and irregularly present in allergic and collagen vascular diseases and malignancies. The hypereosinophilic syndromes cause the highest eosinophil counts, occasionally in the 50,000 to 100,000 per cubic millimeter. Many tissues become infiltrated by eosinophils, a condition leading to organ dysfunction, particularly congestive heart failure. There

was no evidences of drug allergy and toxoplasmosis or other parasitism in this case. The patient had no severe allergic disorders in her past history and no abnormal IgE titer was uncovered when examining her serum. Moreover, there was no infiltration of eosinophils into her muscle and liver. Because of the above reasons, we were able to differentiate eosinophilic polymyositis. Informatively, the specific origin of the patient's eosinophilia could not be determined in other clinical laboratory examinations, and her eosinophilia dissipated after prednisolone therapy.

In conclusion, with reference to this case of polymyositis with PBC and eosinophilia, the Western blotting technique proved to be a very useful tool in reaching diagnosis.

Acknowledgement

The authors thank Special Reference Laboratories for technical assistance.

We are grateful Dr. William E Seaman, University of California, for critically reading the manuscript.

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