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High prevalence of anti-mitochondrial antibodies among patients with some well-defined connective tissue diseases

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SUMMARY

A sensitive enzyme linked immunosorbent assay for determination of low levels of anti-mitochondrial antibodies (AMA) has been developed. With this method, sera from patients with primary biliary cirrhosis (PBC) and patients with different connective tissue diseases were investigated. Ninety percent of PBC sera were found to harbour high levels of AMA and a high proportion of patients with systemic lupus erythematosus (SLE), but also other patients with connective tissue diseases were found to have low affinity or low concentrations of AMA in their sera. AMA positive sera were further investigated with sodium dodecyl sulphate polyacrylamide gel electrophoresis and immunoblotting technique. PBC showed reactivity to 70, 50 and 45 kD mitochondrial polypeptides. SLE sera showed reactivity to 70 and 45 kD polypeptides and furthermore to a 65 kD polypeptide. Many of the AMA positive sera from patients with connective tissue diseases reacted to a 65 kD polypeptide.

Keywords anti-mitochondrial antibodies primary biliary cirrhosis systemic lupus erythematosus connective tissue disease ELISA SDS-PAGE

INTRODUCTION

Anti-mitochondrial antibodies (AMA) were found in sera from patients with certain autoimmune disease states. They were systematized by a combination approach involving indirect immunoflourescence (IIF) on several tissues of human and murine origin and a complement fixation (CF) test using a battery of carefully defined antigens (Meek *et al.*, 1980). The nomenclature M1–M4 was proposed by Berg (1976), M5 by Labro *et al.* (1978), M6 by Homberg *et al.* (1982) and M7 by Klein *et al.* (1984) for each of these antigens.

The M1-antigen is identical to cardiolipin, to which antibodies are produced in cases of syphilis, and also in some cases of systemic lupus erythematosus (SLE) where they show relation to lupus anti-coagulant activity and thrombocytopenia (Harris *et al.*, 1983).

Antibodies against the M2-antigen are found in about 90% of sera from patients with primary biliary cirrhosis (PBC) (Klein, Lindenborg-Fotinis & Berg, 1983) and have so far been the most commonly detected AMA. PBC patients further have AMA of at least one more specificity directed against the adenine nucleotide translocator (ANT)—the only well characterized mitochondrial auto-antigen besides cardiolipin (Schultheiss, Berg & Klingenberg, 1983). M2-antibodies have also been found in sera from patients with a chronic active non-alcoholic HBsAg negative liver disease

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(CAH) showing cholestatic features (Meek *et al.*, 1980). These patients have anti-M4 antibodies in their sera as well (Berg, 1982).

M3-autoantibodies have been detected in sera from patients with drug-induced pseudolupus erythematosus syndrome (PLE) induced by pyrazolone or their metabolites (Sayers, Binder & Berg, 1979).

M5-antibodies have been found in sera from patients with so-called 'undefined collagen disease' (Labro *et al.*, 1978). Most often it has been in patients with SLE and/or autoimmune haemolytic anaemia (AHA). Until now M5-autoantibodies have been detected only in about 2% of patients with SLE (Labro *et al.*, 1978) using IIF and CF techniques.

As might be expected antibodies to native nuclear DNA (dsDNA) in sera from SLE patients have been found to react with mitochondrial dsDNA as well (Reimer *et al.*, 1984).

Anti M6-autoantibodies have been detected in patients with iproniazid-induced hepatitis (Homberg *et al.*, 1982) and recently a partially organ-specific AMA (anti-M7) against heart and kidney mitochondria have been found in sera from patients with cardiomyopathy of unknown origin (Klein *et al.*, 1983).

Non-classified AMA have furthermore been detected in sera from patients with progressive systemic sclerosis (PSS), in a few cases of primary Sjögrens syndrome (SS), rheumatoid arthritis (RA) and polymyalgia rheumatica (PMR) (Gupta *et al.*, 1984; Whaley *et al.*, 1970; Doniach & Walker, 1969; Sattar *et al.*, 1984).

An ELISA technique for AMA determination has recently been developed in our laboratory (Mouritsen *et al.*, 1985). This technique is more sensitive than CF and IIF and no interference exists between the determination of AMA and other autoantibodies to cell constituents since isolated sub-mitochondrial particles (SMP) are the antigenic substrate for the reaction.

The aim of the present work has been to develop a more sensitive ELISA technique to investigate the prevalence of AMA in different well-defined connective tissue diseases. All the previously described ELISA methods have been optimized using relatively high-titred M2-antibodies as reference sera, but we have attempted to detect low affinity AMA as well.

MATERIALS AND METHODS

Sera. Thirty sera were obtained with PBC. The diagnostic criteria have been given elsewhere (Mouritsen *et al.*, 1985). Twenty-nine sera from patients with SLE, 30 sera from RA patients and 10 sera from patients with PSS were taken. The diagnoses of SLE and RA were based on the ARA criteria for these diseases (Roper *et al.*, 1958; Tan *et al.*, 1982). PSS was diagnosed by the criteria of Masi *et al.* (1981).

Forty-eight sera were from patients with SS based on the criteria of Manthorpe *et al.* (1981).

Forty-seven patient sera were from cases of PMR and these cases were diagnosed as described before (Permin, Aldershvile & Nielsen, 1982). Nine sera were from patients with PLE induced by phenytoin, trigion, carbamazepin, hydralazine, propylthiouracil and contraceptive pills. The diagnosis was based on symptoms such as polyarthralgia, myalgia, fever, pulmonary infiltrates, pleurisy, pericarditis and autoimmune phenomena such as anaemia, leukopenia, thrombocytopenia, false positive biological test for syphilis, anti-nuclear antibodies and circulating immune complexes. The clinical and autoimmune abnormalities disappeared after the medication was discontinued.

Finally, 100 sera from healthy blood donors were from the Department of Clinical Immunology, Statens Seruminstitut, Copenhagen.

Antigen. Submitochondrial particles (SMP) were prepared from fresh pig heart muscle as described previously (Mouritsen *et al.*, 1985). Protein concentrations were determined using the Lowry method (Lowry *et al.*, 1951).

ELISA method. Basically the technique was performed as previously described (Mouritsen *et al.*, 1985). Briefly 50 μl of SMP diluted in phosphate buffered saline, pH 7·4 (PBS) was coated onto the ELISA micro-titre plates in concentrations of 20–100 μg protein/ml by overnight incubation at 4°C. Before performing the test, plates were washed three times in washing buffer (PBS containing

0·5 M NaCl and 1% Triton X-100). Sera were diluted in diluting buffer (washing buffer containing 1% bovine serum albumine (BSA) and 0·005% phenol red) and 50 μl were added to each well. After incubation for 1 h at room temperature, the plates were washed extensively and 50 μl of a 1:2500 dilution of peroxidase conjugated rabbit anti-human IgG (DAKOPATTS, Copenhagen) in dilution buffer was added to each well. After another 1 h of incubation at room temperature the plates were washed and peroxidase activity in each well was quantified with ortho-phenyl diamine and H_2O_2 as substrates. The reaction was stopped with 2 M H_2SO_4 after 10 min and the optical density values measured at 492 nm. Sera from patients with connective tissue disorders and from blood donors were tested in two different ELISA methods optimized by testing one high and one low titred AMA positive SLE serum at different serum dilutions. In each ELISA assay a low-titred AMA positive PBC serum was used as positive reference value on each micro-titre plate. The reference serum was diluted 1:1000 in ELISA 1 and 1:100 in ELISA 2 to allow demonstration of an array of AMA affinities in the two ELISA methods. In ELISA 1 the plates were coated with 50 μg SMP protein/ml and sera were diluted 1:100, whereas in ELISA 2 the plates were coated with 20 μg SMP protein/ml and sera were diluted 1:1000. The latter method represents what we previously described as optimal conditions for detection of AMA positive PBC patients. In each assay optical densities (OD) obtained with patient sera were corrected for OD values of control wells containing no serum. All values of patient and donor sera were expressed as a percentage of the OD value of our local positive reference serum which arbitrarily has been given the value of 100 ELISA units/ml. Only a difference of less than 10% on duplicate estimations was accepted. Sera from patients with PBC were only investigated in ELISA 2 because in general it was impossible to detect these high-titred AMA at 1:100 dilutions.

Polyacrylamide gel electrophoresis and immunoblotting. The SMP preparation was analysed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) according to Laemmli (1970) using 0·7 mm gels and 65 υg protein per lane. Electrophoretic transfer to nitrocellulose (Kyhse-Andersen, 1984) and immunoblotting of AMA positive sera was performed by a modified procedure of Towbin, Staehelin & Gordon (1979). Sera and peroxidase conjugates were thus diluted in rabbit serum diluted 1:20 in PBS containing 0·5 M NaCl, 0·05% Tween 20 and 2·5% BSA. This was necessary to avoid non-specific binding of blood donor serum. Sera from patients with connective tissue diseases were diluted 1:50 and sera from PBC patients were diluted 1:160. Control lanes of nitro-cellulose without any serum or without anti-human IgG, respectively, were run in parallel in all experiments.

Inhibition experiments. Serum dilutions (1:100 in PBS) of the AMA positive sera were preincubated for 60 min 37°C, with (a) an excess amount of cardiolipin (Sigma), 1 mg/ml; (b) with native calf thymus DNA (dsDNA) (Sigma), 200 μg/ml; (c) with single stranded DNA (prepared from dsDNA by boiling for 15 min followed by rapid cooling on ice), 200 μg/ml; (d) with affinity purified SS-B (ImmunoVision Inc), 200 μg/ml; (e) with PBS only; and (f) with SMP 200 μg/ml before application to SMP coated micro-titre plates and further testing in the ELISA technique. Anti-dsDNA reactivity was determined by the method described by Holian *et al.* (1975) (Farr assay).

Statistics. The sign-test was used to estimate differences in AMA positivity in the two ELISA assays.

RESULTS

ELISA methods. The sensitivity of ELISA 1 was not further increased by the use of SMP concentrations above 50 μg/ml (Fig. 1). Values above the 99 percentile of AMA values of healthy blood donors were considered positive in the two ELISA methods. ELISA 1: 11/29 SLE sera, 5/30 RA sera, 3/48 SS sera, 3/47 PMR sera, 3/9 PLE sera and 1/10 PSS sera were positive (Fig. 2a). ELISA 2: 1/29 SLE sera, 0/30 RA sera, 2/48 SS sera, 1/47 PMR sera, 0/9 PLE sera, 27/30 PBC sera and 0/10 PSS sera were AMA positive under these conditions (Fig. 2b). None of the AMA negative PBC patients in ELISA 2 became positive in ELISA 1 (data not shown). The difference between the number of AMA positive patients in the two ELISA's were significant ($P < 0·05$). Among the other patients with connective tissue disorders more patients were found positive in ELISA 1, but no significant differences were found compared to ELISA 2. There was however a large type II error due to the low number of AMA positive patients in the individual groups (Fig. 3).

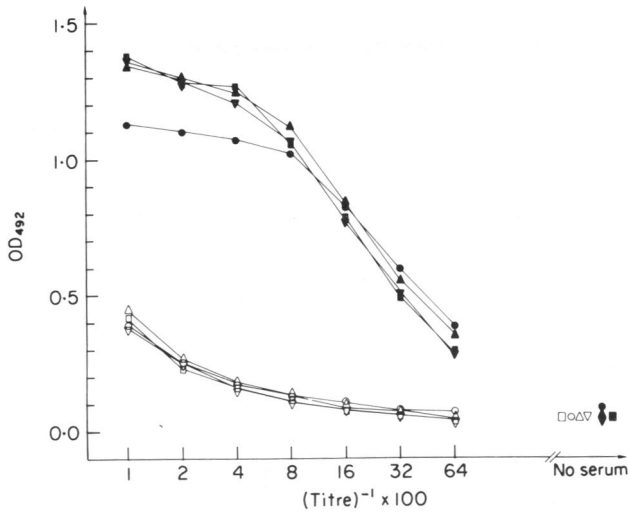

Fig. 1. One high (■) and one low (□) titred AMA positive SLE patient. Antigen concentration in coating solutions: 20 (○), 50 (△), 80 (□) and 100 (▽) μg SMP protein/ml.

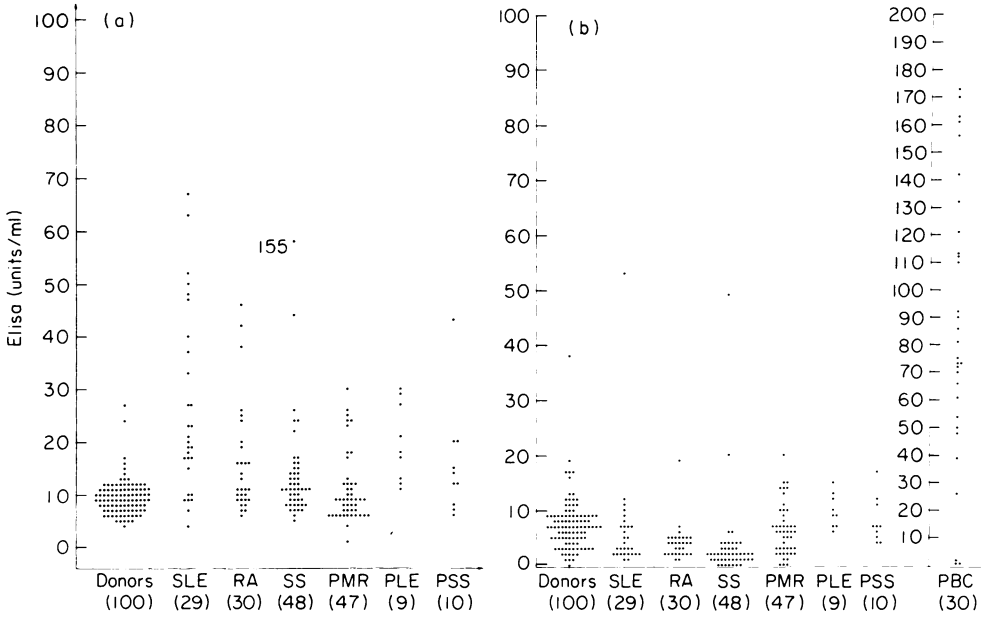

Fig. 2. AMA ELISA-values (units/ml) detected with: (a) ELISA 1 and (b) ELISA 2 in sera from 100 blood donors and a number of patients with different well-defined connective tissue diseases.

Inhibition experiments. None of the investigated AMA positive SLE sera were inhibited by dsDNA, ssDNA or SS-B. Two of the SLE sera were however inhibited by cardiolipin (data not shown). In Fig. 4 results from inhibiting experiments with one high- (SLE 1) and one low-titred (SLE 2) SLE serum are shown. (SS-B inhibition experiments not shown). Only the SMP preparation was able to inhibit IgG binding of all SLE sera to the solid phase (Fig. 4).

SDS-PAGE and immunoblotting. Ten AMA positive PBC sera, ten blood donor sera and all sera

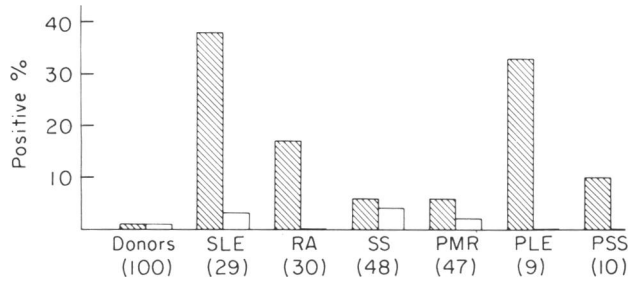

Fig. 3. Percentage of patients positive for AMA in ELISA 1 (▨) and ELISA 2 (□). Values above the 99 percentile based on the study of 100 healthy blood donor sera were considered positive.

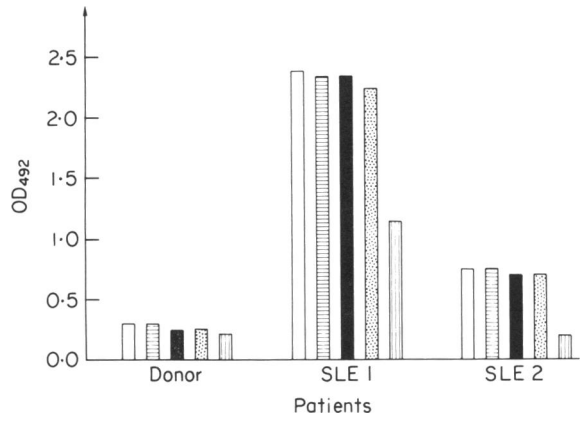

Fig. 4. One AMA negative blood donor serum and two AMA and anti-dsDNA positive sera from patients with SLE. Sera were preincubated with PBS (□), ssDNA (■), dsDNA (■), cardiolipin (▤) and SMP (▥) before testing in ELISA 1.

Table 1. Molecular weights of mitochondrial polypeptides as estimated by SDS-PAGE followed by immunoblotting with sera from patients with PBC and different connective tissue diseases

	Mol. wt of mitochondrial polypeptides			
Disease type	70 kD	65 kD	50 kD	45 kD
PBC	10/10	0/10	6/10	7/10
SLE	6/11	5/11	0/11	4/11
RA	0/5	3/5	0/5	0/5
SS	2/3	0/3	0/3	1/3
PMR	0/3	1/3	0/3	0/3
PLE	0/3	3/3	1/3	0/3
PSS	0/1	1/1	0/1	1/1

found positive in ELISA 1 were investigated. The results are shown in Table 1. All PBC sera reacted with a 70 kD polypedptide, six additionally reacted with a 50 kD peptide and seven reacted with a 45 kD polypeptide (Fig. 5). None of the sera from patients with SLE reacted with the 50 kD polypeptide, whereas a reaction with a 70 kD polypeptide was found in six out of 11 sera and with the 45 kD polypeptide in four out of 11 sera. Additionally one polypeptide band at 65 kD was

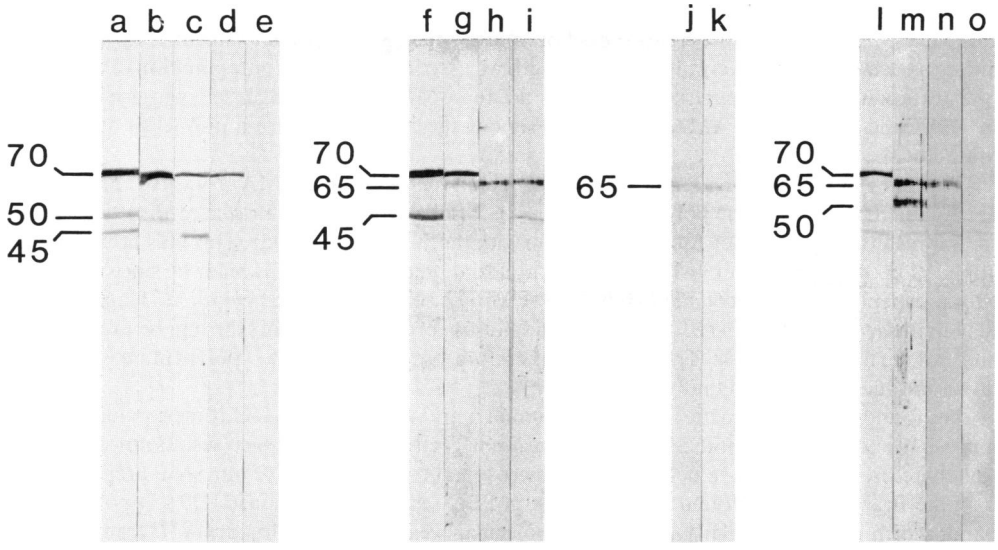

Fig. 5. AMA positive sera from patients and normal blood donor studied with SDS-PAGE and immunoblotting techniques. (a, b, c, d, f) PBC sera; (g, h, i) SLE sera; (j, k) RA sera; (l) SS serum; (m) PLE serum; (n) PMR serum; (e, o) blood donor sera diluted 1:160 and 1:50, respectively.

revealed with 5/11 SLE sera. In two SLE sera the reaction with mitochondrial polypeptides could not be distinguished from the weak 'non-specific' reactions seen in some of the AMA negative blood donors using the same dilutions (Fig. 5). These two patients had ELISA values of 48 and 27 units/ml, respectively. A similar phenomenon was observed with two of the AMA positive RA sera, one PS serum and two PMR sera which all had low ELISA values (25–26 units/ml). Except the SS sera many sera from patients with connective tissue diseases reacted with the 65 kD polypeptide band. At serum dilutions of 1:160 none of the investigated blood donor sera reacted with the mitochondrial polypeptides. At 1:50 3/10 blood donors reacted weakly with multiple polypeptide bands, one of the donors predominantly with the 70 kD band. We chose not to dilute the sera to avoid this 'non-specific' binding because we found it important for the comparision to the reaction pattern of patient sera.

DISCUSSION

This study has shown, that a high proportion of patients with SLE and some patients with other defined connective tissue disorders have low-affinity or low amounts of autoantibodies in their sera directed against mitochondrial proteins. In SLE these antibodies presumably were not directed against cardiolipin or mitochondrial DNA since inhibition experiments were completely negative. The high prevalence of AMA found among patients with SLE in this study was a result of the more sensitive ELISA technique used here. This technique for detecting AMA is at least 20 times more sensitive than the complement fixation technique (Kaplan, Gandolfo & Quaroni, 1984) and the limitations of IIF technique are avoided. Nearly 100% of SLE patients have anti-nuclear antibodies in their serum which may obscure the IIF reading of AMA. When SLE sera have been studied for presence of AMA by ELISA techniques positive control serum usually has been serum for PBC patients (Kaplan *et al.*, 1984; Kenna *et al.*, 1984; Mouritsen *et al.*, 1985). With our ELISA 1 system it was not technically possible to detect most of these high-titred AMA simultaneously with the AMA found in SLE. These low-titred antibodies (Meek *et al.*, 1980) may thus have been missed because of the much stronger signal from the reference AMA found in association with PBC. The number of AMA positive PBC patients found in our present study are in harmony with findings of other

investigators (Christensen *et al.*, 1980; Klein *et al.* 1983). Many patients with SLE have immune complexes in their sera. Binding to mitochondrial proteins through complement is however not likely to occur at the relatively high ionic strength we used in our assay. Contamination of our SMP preparation with nuclear material is unlikely since the AMA content of SLE sera was not inhibited by DNA and only one the AMA positive RA sera was weakly positive for anti-nuclear antibodies (data not shown).

By use of SDS-PAGE and immunoblotting techniques we found AMA from PBC patients to react with 70, 50 and 45 kD polypeptides (Fig. 5). Parallel to our work Frazer *et al.* (1985) found similar results, although they found no reaction with the 50 kD polypeptide. This may be due to the application of protease inhibitors in the mitochondrial preparations of Frazer *et al.* since reactions with polypeptides at about 50 kD (51–55 kD) recently have been reported by others (Ishii, Saifuku & Namihisa, 1985; Manns *et al.*, 1985) who did not use protease inhibitors during the preparation and storage of mitochondria. The possibility therefore exists that the 50 kD polypeptide is a break-down product of the 70 kD polypeptide.

We found very low activities of the microsomal markers NADPH-cytochrome c reductase and glucose-6-phosphatase in both SMP and microsomal fractions from pig heart (results not shown). It was therefore not possible to determine the microsomal contamination of our SMP preparations.

Like Frazer *et al.* we found no antibodies in PBC sera directed against a 30 kD polypeptide band, which is the molecular, which is the molecular weight reported for the ATP translocating protein (Klingenberg, Riccio & Aquila, 1978) which has been postulated to be a partially organ specific auto-antigen in PBC (Schultheiss *et al.*, 1983). These investigators further were able to inhibit the function of the reconstituted ATP translocating protein from rat liver when using carboxyatractylate as a protecting agent during the preparation procedures (Schultheiss *et al.*, 1984). Neither Frazer nor we used this agent when performing SDS-PAGE on the SMP preparation. Schultheiss *et al.* (1983) moreover found 10 out of 13 PBC sera anti-ANT negative when using ANT isolated from beef heart, which may explain the missing reactivity with a 30 kD protein using pig heart mitochondria.

We did not find any reactivity with the 50 kD polypeptide in SLE sera but some sera were seen to react with a 65 kD polypeptide. With a few of the AMA positive sera exhibiting predominantly low ELISA values, no specific reaction against any of the polypeptide bands could be delineated. This is undoubtedly because the immunoblotting technique only allows a semiquantitative estimation of the strength of the reaction.

It is tempting to speculate if the 'non-specific' reactivity to mitochondrial polypeptides found in some donor sera at 1:50 dilutions and maybe the specific reactions found in patient sera may be cross-reacting antibodies to bacterial proteins. There is accumulating evidence for the concept that mitochondria arose as bacterial endosymbionts within some ancestral type of eukaryotic cell (Yang *et al.*, 1985; Gray *et al.*, 1982).

Whether the 65 kD polypeptide is identical to the M5 antigen remains to be established, but like Ishii *et al.* (1985), Manns *et al.* (1985) and Frazer *et al.* (1985) we have shown that serum from the same patient may react with different polypeptides or at least with different affinities to these polypeptides.

Reaction with cardiolipin (Norberg *et al.*, 1984) is not likely to be seen since the molecular weight is too small to be detected in SDS-PAGE and immunoblotting techniques used here. Earlier we likewise found no reaction of syphilis sera with our antigen (Mouritsen *et al.*, 1985) probably because the detergents used in our buffer are prone to extract the cardiolipin from the SMP. The finding that two of the AMA positive SLE sera were inhibited by cardiolipin coincides with the very recent finding of Meroni *et al.* (1986) that high concentrations of cardiolipin are able to decrease the titres of anti-M5 positive sera.

Till now we have only investigated mitochondrial preparations from pig heart. Mitochondria from different organs need to be examined and the relationship between the M5-antigen and the 65 kD polypeptide has to be clarified by investigation of well characterized mitochondrial membrane subfractions. We have not yet examined associations between the presence of low affinity AMA in sera from SLE patients and clinical features. These studies are now in progress.

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REFERENCES

BERG, P.A. (1976) Chronish-aktive (aggressive) Hepatitis (zur differentialdiagnosen der Lupoiden (ANA-positiven) und cholestatisch verlaufenden (AMA-positiven) Hepatitis). *Dtsch. Med. Wochenschr.* **101,** 1536.

BERG, P.A. (1982) Klinik und Immunologie der autoimmunen chronisch aktiven Hepatitis und der primär-biliären Zirrhose. *Immun. Infekt.* **10,** 3.

CHRISTENSEN, E., CROWE, J., DONIACH, D., POPPER, H., RANEK, L., RODES, J., TYGSTRUP, N. & WILLIAMS, R. (1980) Clinical pattern and course of disease in primary biliary cirrhosis based on an analysis of 236 patients. *Gastroenterol.* **78,** 236.

DONIACH, D. & WALKER, J.G. (1969) A unified concept of autoimmune hepatitis. *Lancet*, **i,** 813.

FRAZER, I.H., MACKAY, I.R., JORDAN, T.W., WHITTINGHAM, S. & MARZUKI, S. (1985) Reactivity of anti-mitochondrial autoantibodies in primary biliary cirrhosis: definition of two novel mitochondrial polypeptide autoantigens. *J. Immunology*, **135,** 1739.

GRAY, M.W. & DOOLITTLE, W.F. (1982) Has the endosymbiont hypothesis been proven? *Microbiol. Rev.* **46,** 1.

GUPTA, R.C., SEIBOLD, J.R., KRISHAN, M.R. & STEIGERWALD, J.C. (1984) Precipitating autoantibodies to mitochondrial proteins in progressive systemic sclerosis. *Clin. exp. Immunol.* **58,** 68.

HARRIS, E.N., BOEY, M.L., MACKWORTH-YOUNG, C.G., GHARAVI, A.E., PATEL, B.M., LOIZOO, S. & HUGHES, G.R.V. (1983) Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet*, **ii,** 1211.

HOLIAN, J., GRIFFITHS, J.D., GLASS, D.N., MAINI, R.N. & SCOTT, J.T. (1975) Standardised assay for measurement of antibodies to DNA. *Scand. J. Rheumatology*, **4,** 8.

HOMBERG, J.C., STELLY, N., ANDREIS, I., ABUAF, F., SAADOUN, F. & ANDRE, J. (1982) A new antimitochondrial antibody (anti-M6) in iproniazid-induced hepatitis. *Clin. exp. Immunol.* **47,** 93.

ISHII, H., SAIFUKU, K. & NAMIHISA, T. (1985) Multiplicity of mitochondrial inner membrane antigens from beef heart reacting with anti-mitochondrial antibodies in sera of patients with primary biliary cirrhosis. *Immun. Lett.* **9,** 325.

KAPLAN, M.M., GANDOLFO, J.V. & QUARONI, E.G. (1984) An enzyme-linked immunosorbent assay (ELISA) for detecting antimitochondrial antibodies. *Hepatology*, **4,** 727.

KENNA, J.G., NEUBERGER, J., DAVIES, E., EDDLESTON, A.L.W.F. & WILLIAMS, R. (1984) A simple enzyme-linked immunosorbent assay for detection of anti-mitochondrial antibodies. *J. Immunol. Meth.* **73,** 401.

KLEIN, R., MAISCH, B., KOCHSIEK, K. & BERG, P.A. (1984) Demonstration of organ specific antibodies against heart mitochondria (anti-M7) in sera from patients with some forms of heart disease. *Clin. exp. Immunol.* **58,** 283.

KLEIN, R., LINDENBORN-FOTINIS, J. & BERG, P.A. (1983) Use of ATPase-associated antigen (M2) for detection of antimitochondrial antibodies in primary biliary cirrhosis by fluorometric immunoassay. *J. Immunol. Meth.* **64,** 227.

KLINGENBERG, M., RICCIO, P. & AQUILA, H. (1978) Isolation of the ADP, ATP carrier as the carboxyatractylate protein complex from mitochondria. *Biochem. Biophys. Acta.* **503,** 193.

KYHSE-ANDERSEN, J. (1984) Electroblotting of multiple gels: a simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. *J. Biochem. Biophys. Meth.* **10,** 203.

LABRO, M.T., ANDRIEU, M-C., WEBER, M. & HOMBERG, J.C. (1978) A new pattern of non-organ and non-species specific anti-organelle antibody detected by immunofluorescence: the mitochondrial antibody number 5. *Clin. exp. Immunol.* **31,** 357.

LAEMMLI, U.K. (1970) Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature*, **227,** 680.

LOWRY, O.H., ROSEBROUGH, M.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the folin phenol reagent. *Biol. Chem.* **193,** 265.

MANNS, M., GERKEN, G., MEUER, S., PORALLA, T. & MEYER ZUM BÜSCHENFELDS, K.H. (1985) Characterization of primary biliary cirrhosis (PBC) specific mitochondrial determinants by immunoblotting. *J. Hepatology*, **1,** 85.

MANTHORPE, R., FROST-LARSEN, K., ISAGER, H. & PRAUSE, J.U. (1981) Sjogren's syndrome. *Allergy*, **36,** 139.

MASI, A.T., RODNAN, G.P. & MEDSGER, T.A. (1981) Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Bull. Rheum. Dis.* **31,** 1.

MEEK, F., KHOURY, E.L., DONIACH, D. & BAUM, H. (1980) Mitochondrial antibodies in chronic liver diseases and connective tissue disorders: further characterization of the autoantigens. *Clin. exp. Immunol.*

MERONI, P.L., HARRIS, E.N., BRUCATO, A., TICANI, A., BARCELLINI, W., GHARAVI, A.E., BALESTRIERI, G., HUGHES, G.R.V. & ZANUSSI, C. (1986) Anti-mitochondrial type M5 and anti-cardiolipin antibodies in autoimmune disorders: studies on their association and crossreactivity. *2nd World Symposium on Anti-phospholipid Antibodies.* St Thomas's Hospital, London, 4 April 1986.

MOURITSEN, S., HØIER-MADSEN, M., DEMANT, E.J.F.

& PERMIN, H. (1985) Enzyme-linked immunosorbent assay for determination of anti-mitochondrial antibodies. *Acta. path microbiol. Scand. Sect. C.* **93,** 205.

NORBERG, R., GLRDLUND, B., THORSTENSSON, R. & LIDMAN, K. (1984) Further immunological studies of sera containing anti-mitochondrial antibodies, type M5. *Clin. exp. Immunol.* **58,** 639.

PERMIN, H., ALDERSHVILE, J. & NIELSEN, J.O. (1982) Hepatitis B virus infection in patients with rheumatic diseases. *Ann. Rheum. Dis.* **41,** 479.

REIMER, G., RUBIN, R.L, KATZEN, B.L. & TAN, E.M. (1984) Anti-native DNA antibodies from autoimmune sera also bind to DNA in mitochondria. *J. Immunol.* **133,** 2532.

ROPER, M.V., BENNETT, G.A., COBB, S., JACOX, R. & JESSAR, R.A. (1958) Revision of diagnostic criteria for rheumatoid arthritis. *Bull. Rheum. Dis.* **9,** 175.

SATTAR, M.A., CAWLEY, M.I.D., HAMBLIN, T.J. & ROBERTSON, J.C. (1984) Polymyalgia rheumatica and antimitochondrial antibodies. *Ann. rheum. Dis.* **43,** 264.

SAYERS, T.J., BINDER, T. & BERG, P.A. (1979) Heterogeneity of anti-mitochondrial antibodies: characterization and separation of the antigen associated with the pseudolupus erythematosus syndrome. *Clin. exp. Immunol.* **37,** 68.

SCHULTHEISS, H.P., BERG, P.A. & KLINGENBERG, M. (1983) The mitochondrial adenine nucleotide translocator is an antigen in primary biliary cirrhosis. *Clin. exp. Immunol.* **54,** 648.

SCHULTHEISS, H.P., BERG, P.A. & KLINGENBERG, M. (1984) Inhibition of the adenine nucleotide translocator by organ specific autoantibodies in primary biliary cirrhosis. *Clin. exp. Immunol.* **58,** 516.

TAN, E.M., COHEN, A.S. & FRIES, J. (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25,** 1271.

TOWBIN, H., STAEHELIN, T. & GORDON, J. (1979) Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. natn. Acad. Sci.* **6,** 4350.

WHALEY, K., WILLIAMSON, J., DICK, W.C., GOUDIE, R.B., NUKI, G. & BUCHANAN, W.W. (1970) Liver disease in Sjögren's syndrome and rheumatoid arthritis. *Lancet*, **ii,** 861.

YANG, D., OYAIZU, Y., OYAIZU, H., OLSEN, G.J. & WOESE, C.R. (1985) Mitochondrial origins. *Proc. natn. Acad. Sci.* **82,** 4443.