CONCISE

PUBLICATIONS

Antinuclear Antibody with Distinct Specificity for Polymyositis

J. FREDERICK WOLFE, EDWARD ADELSTEIN, and GORDON C. SHARP

From the Department of Medicine, University of Missouri Medical Center, and the Department of Pathology, Harry S. Truman Veterans Administration Hospital, Columbia, Missouri 65201

ABSTRACT In the course of studying antinuclear antibodies in the rheumatic diseases, a new precipitin reaction (provisionally referred to as PM-1) was observed between calf thymus nuclear extract and polymyositis sera. Objectives of this study were to further define the immunologic nature of this reaction and to determine its specificity for polymyositis. Immunodiffusion studies using calf thymus nuclear extract revealed the PM-1 precipitin line in 17 of 28 patients with polymyositis. This reaction was not produced by sera of 460 patients with other diseases. Enzyme and heat treatments of the nuclear extract showed that PM-1 was distinct from native DNA, ribonucleoprotein, and Sm antigens. Fractionation of PM-1-positive serum by 30% ammonium sulphate and Sephadex G-200 chromatography revealed that the factor producing the PM-1 precipitin reaction was in a serum fraction which showed only IgG by immunoelectrophoresis against anti-whole human serum. Because of the apparent strong specificity, the PM-1 system may represent a marker antibody for polymyositis.

INTRODUCTION

Serological findings, sufficiently sensitive and specific, have provided useful diagnostic tools in certain of the rheumatic diseases. The findings of circulating antibody to native DNA help to establish a diagnosis of systemic lupus erythematosus (1), and antibody to nuclear ribonucleoprotein (RNP)[1] is helpful in the diagnosis of mixed connective tissue disease (2). In polymyositis, however, there has been no consistent serological finding which is sufficiently sensitive and specific to be useful as a diagnostic test.

Fluorescent antinuclear antibody tests have been found to be positive to a variable degree, ranging from 6 to 30% in different studies of polymyositis (3, 4). However, the specific antigen involved in the nuclear staining was not identified in these studies. In another study, an IgG antibody which reacted with capillary walls in mouse kidney was detected in the sera of patients with a number of rheumatic diseases including dermatopolymyositis (5). In the course of studying the correlation of various antinuclear antibodies with clinical characteristics in the rheumatic diseases, we have observed a new precipitin reaction with calf thymus nuclear extract which appears to be specific for polymyositis-dermatomyositis.

METHODS

Sera of patients were collected during hospitalization or clinic visits at this and other medical centers and frozen in small aliquots before testing. A crude calf thymus nuclear extract (CTNE) (6), DNA (7), and extractable nuclear antigen (2) were prepared according to published methods. Native DNA and extractable nuclear antigen were used in passive hemagglutination (1).

This work was presented in part at the Meeting of the American Federation for Clinical Research, Atlantic City, N. J., 2 May 1976 (1976. *Clin. Res.* **24**: 49A.) and at the American Rheumatism Association Meeting in Chicago, Ill., 11 June 1976.

Received for publication 12 August 1976 and in revised form 7 October 1976.

[1] *Abbreviations used in this paper:* CTNE, Calf thymus nuclear extract; RNP, ribonucleoprotein.

Double diffusion was performed in 0.4%. Agarose (Sea-Kem Irish Moss Extractives, Springfield, N. J.) as previously described (6). After plates had been incubated at 23°C and read at 24 h, they were placed at 4°C and observed again at 48, 72, and 96 h. Sera containing antibodies specific for RNP, Sm, native, and denatured DNA were placed in adjacent wells to sera being tested to characterize the immune precipitates which resulted. Positive sera were also tested in immunodiffusion with CTNE which had been treated with deoxyribonuclease, ribonuclease, or trypsin, or exposed to 37 or 56°C according to published methods (6). Immunoelectrophoresis using anti-whole human serum, fractionation of gamma globulin by 30% ammonium sulfate precipitation, and Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) chromatography, and fluorescent antinuclear antibody tests were performed by standard laboratory methods.

Diagnosis of polymyositis was made on the basis of: (a) proximal muscle pain and (or) weakness involving pelvic girdle, pectoral girdle, and (or) neck flexors; (b) elevation of serum creatine phosphokinase and aldolase (c) electromyogram showing myopathic changes; and (d) muscle biopsy showing inflammatory infiltrates and (or) muscle necrosis. Patients were classified as dermatomyositis if they had the above criteria and characteristic skin changes. Patients were classified as polymyositis-scleroderma overlap if they had skin changes consistent with scleroderma in addition to the findings of polymyositis. Using these criteria, 28 patients were classified as having a polymyositis syndrome.

RESULTS

Fig. 1 shows the line of precipitation, provisionally referred to as PM-1, which formed between the CTNE and the sera of patients with polymyositis. Because the PM-1 precipitin line is rather weak, it is difficult to appreciate in a reproduction but it has clearly been observed that the PM-1 line crosses both the RNP and the Sm lines, indicating that the PM-1 antigen is distinct from these antigens. It was often necessary to observe plates for 48, 72, or 96 h to see PM-1 precipitates form and establish their identity. The reactions of all PM-1-positive sera showed lines of identity. Table I lists various enzymatic and physical treatments performed on the CTNE and their effect on the precipitin reactions, which further demonstrates that PM-1, RNP, and Sm are different antigens. The PM-1 antigen was sensitive to trypsin and heating at 56°C for 1 h, but was resistant to DNase and RNase digestion. Fractionation of PM-1-positive serum by 30% ammonium sulfate precipitation and Sephadex G-200 chromatography revealed that the factor producing the PM-1 precipitin reaction was in a serum fraction which showed only IgG by immunoelectrophoresis against anti-whole human serum.

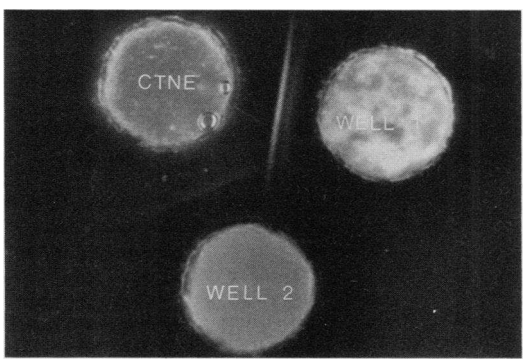

FIG. 1 The well-marked CTNE contains calf thymus nuclear extract. Well 1 contains serum from a patient with antibodies to RNP and Sm. Well 2 contains serum from a patient with polymyositis which produces the PM-1 line that is distinct from both the RNP and Sm lines.

TABLE I
Characterization of Nuclear Extract Antigens

Treatment of CTNE	PM-1	Sm	RNP
RNase	Resistant	Resistant	Sensitive
Dnase	Resistant	Resistant	Resistant
37°C-6 h	Resistant	Resistant	Sensitive
56°C-1 h	Sensitive	Resistant	Sensitive
Trypsin	Sensitive	Partially sensitive	Sensitive

As shown in Table II, 17 of 28 patients with polymyositis, dermatomyositis, or polymyositis-scleroderma overlap (61%) were positive for the PM-1 reaction. Serial studies over a 5-yr period on 10 of these polymyositis patients, 5 positive and 5 negative for the PM-1 reaction, showed that if PM-1 was present, it remained whether or not the disease was active or in remission. Likewise, if PM-1 was not present at the time the patient was first seen it remained absent regardless of the clinical course. Other groups of patients studied including 169 with mixed connective tissue disease, 100 with systemic lupus erythematosus, 30 with scleroderma, 30 with rheumatoid arthritis, 10 with osteoarthritis, 21 with other rheumatic diseases, and 100 other hospitalized patients were uniformly negative for the PM-1 precipitin reaction.

Fluorescent antinuclear antibody tests were performed on all 28 patients with polymyositis. 8 of the 17

TABLE II
Incidence of PM-1 in Polymyositis Syndromes

Clinical syndrome	Positive total	%
Polymyositis	9/14	64
Dermatomyositis	1/6	17
Polymyositis-scleroderma overlap	7/8	87
Total	17/28	61

sera positive for PM-1 were positive in fluorescent antinuclear antibody tests with titers ranging from 1:20 to 1:80, usually showing a speckled pattern. These eight positive sera were from four patients with polymyositis and four with polymyositis-scleroderma overlap. Only 2 of 11 sera negative for the PM-1 reaction were positive, both with a titer of 1:20.

DISCUSSION

The present study and a recent report by Reichlin and Mattioli (8) suggest that a particular antinuclear antibody may be a highly specific serological marker for polymyositis-dermatomyositis. Because they found the precipitin reaction to be weak and sporadic, Reichlin and Mattioli employed an indirect test in which Fab fragments of sera being tested were studied for their capacity to produce significant inhibition of complement fixation by a reference serum reacting with CTNE. In their study, 10 of 17 (59%) polymyositis sera and 3 of 84 sera from normals or patients with other diseases produced significant inhibition. Although the PM-1 precipitin reaction is not strong, we have found it to be consistent and reproducible. The immunodiffusion method has the advantage that we can demonstrate directly that PM-1 reactions of all positive sera show lines of identity. Even though immunodiffusion is insensitive, we were able to show the PM-1 reaction in 17 of 28 (61%) patients with polymyositis or dermatomyositis but did not find the precipitin line in any of 460 sera from patients with other diseases.

It is possible, because of the similarity in physiochemical characteristics of the antigen, that the same system is being described in these two independent investigations. However, the studies differ with respect to the incidence of the antibody in patients with dermatomyositis. We are presently pursuing this question through an exchange of sera.

It is not clear at this time whether the PM-1-positive and PM-1-negative patients represent different clinical subgroups of polymyositis. No definite clinical distinctions are apparent to us at this time. Thus, our finding that 40% of polymyositis patients are negative for PM-1 may simply be due to the insensitivity of the methods used in detection.

Because muscle-like elements have been described in thymus (9), it is conceivable that the PM-1 antigen could be a component of such structures and may not necessarily be derived from thymocytes or other immunologic cells in thymus. The PM-1 antigen is definitely distinct from RNP, Sm, and DNA antigens. Since it is sensitive to trypsin, it may be a nuclear protein. Further studies are in progress to define this antigen and to determine whether the immunologic reaction is thymus specific.

ACKNOWLEDGMENTS

We thank Mrs. Meridith Manning and Miss Cindy Evers for their skilled technical assistance and Mrs. Linda O'Connor, Miss Debbie Harman, and Mrs. Nanci Hubbard for their secretarial assistance.

This research was supported in part by a grant from the U. S. Public Health Service (AM 16141), a grant from The Arthritis Foundation, and a Clinical Research Center grant (RR-00-287-10).

REFERENCES

1. Sharp, G. C., W. S. Irvin, R. L. LaRoque, C. Velez, V. Daly, A. D. Kaiser, and H. R. Holman. 1971. Association of autoantibodies to different nuclear antigens with clinical patterns of rheumatic disease and responsiveness to therapy. *J. Clin. Invest.* **50**: 350–359.
2. Sharp, G. C., W. S. Irvin, E. M. Tan, R. G. Gould, and H. R. Holman. 1972. Mixed connective tissue disease—an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am. J. Med.* **52**: 148–159.
3. Stern, G. M., A. L. Rose, and K. Jacobs. 1967. Circulating antibodies in polymyositis. *J. Neurol. Sci.* **5**: 181–183.
4. Wier, D. M., E. J. Holborow, and G. D. Johnson. 1961. A clinical study of serum antinuclear factor. *Br. Med. J.* **1**: 933–937.
5. Tan, E. M., and C. M. Pearson. 1972. Rheumatic disease sera reactive with capillaries in the mouse kidney. *Arthritis Rheum.* **15**: 23–28.
6. Northway, J. D., and E. M. Tan. 1972. Differentiation of antinuclear antibodies giving speckled staining patterns in immunofluorescence. *Clin. Immunol. Immunopathol* **1**: 140–154.
7. Kay, E. R. M., N. S. Simmons, and A. L. Dounce. 1952. An improved preparation of sodium desoxyribonucleate. *J. Am. Chem. Soc.* **74**: 1724–1726.
8. Reichlin, M. and M. Mattioli. 1976. Description of a serological reaction characteristic of polymyositis. *Clin. Immunol. Immunopathol.* **5**: 12–20.
9. Goldstein, G., and I. R. Mackay. 1969. Structure and development of the human thymus. *In* The Human Thymus. Warren H. Green, Inc., St. Louis, Mo. 1st edition. 1–36.