JCI The Journal of Clinical Investigation

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J Clin Invest. 1979;64(3):820-823. https://doi.org/10.1172/JCI109529.

Research Article

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Characterization of a Distinct Nuclear Acidic Protein Antigen (MA) and Clinical Findings in Systemic Lupus Erythematosus Patients with MA antibodies

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ABSTRACT Circulating antibodies against certain nuclear acidic protein antigens have been shown to have diagnostic and prognostic importance in connective tissue disease. We describe a new precipitin system found in the sera of patients with systemic lupus erythematosus. The antigen, called MA, was prepared from calf thymus nuclei, and was shown to be distinct from other nuclear acidic protein antigens by physicochemical and immunologic techniques. MA antibodies were detected in the serum of 12 of 66 lupus patients and in none of 554 sera from normal controls or patients with other rheumatic diseases. Lupus patients having MA antibodies had more severe disease than did lupus patients with Sm or native DNA antibodies, manifested by recalcitrant skin rashes and a significantly greater incidence of hypocomplementemia, serious renal disease, hypertension, hepatosplenomegaly, lymphadenopathy, and neurological disease (P values range from 0.025 to 0.005). The presence of circulating MA antigen was demonstrated in three lupus patients immediately before a flare of nephritis. These data suggest that MA is a nuclear acidic protein antigen that may identify a subset of lupus patients with very severe disease. The presence of the antigen in the circulation before clinical flares suggests a possible biologic role for the MA system in an immune complex nephritis.

INTRODUCTION

Circulating antibodies to native DNA (n-DNA)[1] and certain nuclear acidic protein antigens such as Sm, nuclear ribonucleoprotein (n-RNP), PM-1, SS-A, and SS-B have been shown to have diagnostic and prognostic importance in connective tissue diseases (1–5). Antibodies to n-DNA, in particular, play a significant biologic role in an immune complex nephritis in patients with systemic lupus erythematosus (SLE) (6). We describe in this report the finding of circulating antibody to a distinct new nuclear acidic protein antigen, provisionally called MA, which may be specific for a subset of SLE patients with particularly severe disease.

METHODS

Description of patients studied. Patient sera referred from the University of Missouri Medical Center and other medical centers were tested for antibodies to MA, PM-1, Sm, n-RNP, and n-DNA. After the antibody pattern was characterized, a modified American Rheumatism Association (ARA) database form was completed, documenting the clinical and laboratory parameters of the patient's disease. Clinical data were analyzed on a PDP-12 mini computer (Digital Equipment Corp., Marlboro, Mass.). All of the patients with MA antibodies and the majority of patients with Sm or n-DNA antibodies in this study fulfilled at least 4 out of the 14 preliminary ARA criteria for the diagnosis of SLE (7). Statistical evaluation was performed with chi-square analysis.

Renal disease was said to be present if the patient had granular or cellular casts present, proteinuria of >200 mg/24 h, or an abnormal renal biopsy. Neurological disease included seizures, coma, or organic brain syndromes in the absence of other known causes and presumably because of the underlying disease process.

Immunologic testing. Calf thymus nuclear extract and DNA antigens used for the immunologic studies were prepared according to published techniques (2). The calf thymus nuclear extract was treated by buffer dialysis to establish pH sensitivity of the various nuclear antigens (8) and further tested for susceptibility to digestion with RNase, DNase, and trypsin (Worthington Biochemical Corp., Freehold, N. J.) (2) and for heat lability (8). Immunodiffusion with 0.4% agarose was used for detection of circulating nuclear antigen and antibodies. Sera containing antibodies specific for n-RNP, Sm, and PM-1 were placed in wells adjacent to wells of sera being tested to characterize the immune precipitates that developed. Plates were incubated at room temperature and the MA precipitin occurred within the first 24 h. Passive

Received for publication 15 March 1979 and in revised form 4 May 1979.

[1] *Abbreviations used in this paper*: n-DNA, native DNA; n-RNP, nuclear ribonucleoprotein; SLE, systemic lupus erythematosus.

hemagglutination (2), counterimmunoelectrophoresis (9), and the *Crithidia luciliae* assay (10) were also used to detect the presence of Sm, n-RNP, and n-DNA antibodies. Immunoglobulin (Ig)G purification from a serum positive for MA antibody was achieved by the use of affinity chromatography. Rabbit anti-human IgG was attached to activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N. J.), and IgG containing MA antibodies was then eluted with 0.1 M sodium thiocyanate (11). The purity of the immunoglobulin preparation was confirmed by immunoelectrophoresis. The fluorescent antinuclear antibody test was performed according to standard techniques using rat liver as substrate (12).

RESULTS

Fig. 1 shows that the immune precipitate that formed with the MA antigen crossed the precipitin lines that formed with the Sm, n-RNP, and PM-1 nuclear antigens. Similar immunodiffusion tests, kindly performed by Dr. Margaret Alspaugh of the Louisiana State University, New Orleans, La., showed nonidentity of MA with the SS-B, SS-A, RAP, and SCL-1 nuclear antigens. A serum exchange with Dr. Eng Tan of the University of Colorado, Denver, Colo. confirmed the nonidentity of the MA and the soluble nucleoprotein antigen systems (13). Thus, the MA antigen is immunologically distinct from other nuclear acidic protein antigens. Treatment of the nuclear extract with RNase and DNase did not affect the immune reactivity of the MA system, but digestion with trypsin abolished the reaction. The MA antigen migrated toward the anode in immunoelectrophoresis. The physiocochemical characteristics of MA in comparison to other nuclear acidic protein antigens are summarized in Table I. Again, MA antigen appears to be distinct from other nuclear antigens previously described.

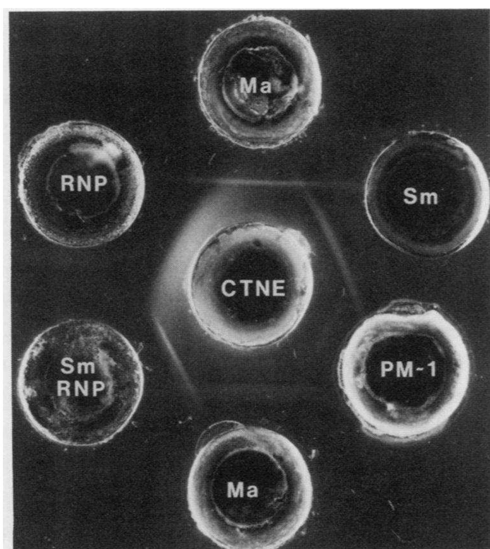

FIGURE 1 The immune precipitate produced by the reaction of antigen, calf thymus nuclear extract in the center well, and a patient's serum containing MA antibodies crosses the immune precipitates of Sm, n-RNP, and PM-1, demonstrating the nonidentity of these antigens.

Antibody nature of the MA precipitin. Pure IgG was prepared by affinity chromatography from a patient's serum that was monospecific for MA antibody. When this IgG fraction was used in immunodiffusion, it produced a single precipitin band identical with that produced by the whole serum containing the MA antibody. Thus, we believe the serum reactant to be an immunoglobulin, and therefore an antibody.

Specificity of the MA system for SLE. The MA antibody appears to be highly specific for SLE. Of 67 rheumatoid arthritis sera; 52 mixed connective tissue disease sera; 61 polymyositis sera; 108 miscellaneous sera including degenerative joint disease, gout, and pseudogout; 17 normal control sera; and 346 SLE sera; only 12 patients had MA antibodies, and all 12 had SLE.

Serologic pattern of patients with MA antibodies. All patients with MA antibodies had a positive test for fluorescent antinuclear antibody, and all but one of these patients had a distinctive pattern of immunofluorescence as shown in Fig. 2. The fluorescent staining occurred in large speckles located at the periphery of the nucleus of the cell. Other antinuclear antibodies found in the sera of patients with MA antibodies included 4 patients with Sm antibodies, 7 patients with n-RNP antibodies, and 10 patients with n-DNA antibodies. However, those patients with n-DNA antibodies almost always had low titers of the antibodies, regardless of the technique used for their detection.

Clinical parameters. The 12 patients with MA antibodies were compared to groups of SLE patients having Sm and n-RNP antibodies (n = 96) and only DNA antibodies (n = 56) as shown in Table II. Patients with MA antibodies had a significantly higher frequency of hypocomplementemia, splenomegaly, lymphadenop-

TABLE I
Comparison of Physicochemical Characteristics of MA Antigen with Other Nuclear Acidic Protein Antigens

Antigen treatment	MA	Sm	n-RNP	PM-1	SS-B	SS-A
RNase	R	R	S	R	R	R
DNase	R	R	R	R	R	R
Trypsin	S	PS	S	S	S	S
37°C, 6 *h*	R	R	S	R	S	—
56°C, 6 *h*	S	R	S	S	S	R(1 h)
pH stability*	3–8	4–10	4–8	3–11	6–10	—

Abbreviations used in this table: R, resistant; S, sensitive; PS, partially sensitive.
* The antigen was subjected to acid and alkali buffer dialysis, and pH stability refers to the pH range in which the antigen maintained its antigenicity as tested by immunodiffusion.

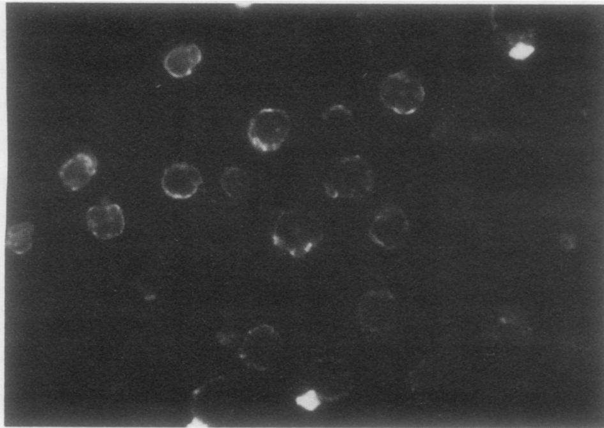

FIGURE 2 The photograph demonstrates the immunofluorescent pattern showing the distinct peripheral location of the speckled nuclear staining in patients with MA antibodies.

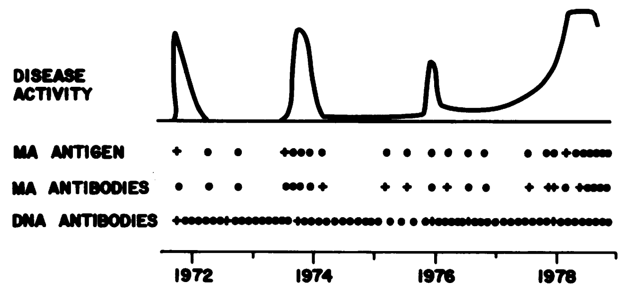

FIGURE 3 The disease course and serologic characteristics of the proband case are illustrated in this figure. Each disease flare was manifested by glomerulonephritis, bullous skin rash, hypocomplementemia, and anemia. Circulating MA antigen was detected before three of the clinical flares, and alternated in its presence with MA antibodies.

athy, renal disease, hepatomegaly, hypertension, and neurological involvement, and a lower frequency of Raynaud's phenomenon and sclerodactyly when compared with patients with Sm antibodies. Hypocomplementemia, fever, splenomegaly, lymphadenopathy, and hepatomegaly were more frequent in patients with MA antibodies than in patients with DNA antibodies. Raynaud's phenomenon was less frequent in patients with MA antibodies than in patients with only DNA antibodies. Recalcitrant lupus skin rashes and marked lymphadenopathy were also particularly characteristic of the MA group.

TABLE II
Comparison of the Prevalence of Certain Clinical Characteristics in Three Groups of SLE Patients Differing in their Serologic Pattern

	MA antibodies $n = 12$	Sm and n-RNP antibodies $n = 96$	n-DNA antibodies $n = 56$
	%	%	%
Hypocomplementemia	83	53 $P < 0.005$*	60 $P < 0.025$‡
Fever	83	52 NS	43 $P < 0.025$
Splenomegaly	75	23 $P < 0.005$	17 $P < 0.005$
Lymphadenopathy	75	23 $P < 0.005$	17 $P < 0.005$
Renal disease	75	41 $P < 0.01$	57 NS
Hepatomegaly	58	17 $P < 0.005$	13 $P < 0.005$
Hypertension	50	17 $P < 0.05$	28 NS
Neurological involvement	42	14 $P < 0.025$	37 NS
Raynaud's phenomenon	0	57 $P < 0.005$	35 $P < 0.025$
Sclerodactyly	0	29 $P < 0.05$	14 NS

* P values in this column compare patients with MA and Sm plus n-RNP antibodies.
‡ P values in this column compare patients with MA and n-DNA antibodies.

Circulating MA antigen and antibody. The disease course and serologic characteristics of the proband MA case are shown in Fig. 3. Each disease flare shown by the peaks at the top of the graph was manifested by severe hypocomplementemia, glomerulonephritis, anemia, and bullous and vesicular skin lesions. Circulating MA antigen was detected on three separate occasions just before a clinical flare. MA antibody first appeared 20 mo after MA antigen was first detected and alternated in its presence with MA antigen thereafter. The alternating presence of the MA antigen and antibody has been observed in two other patients as well.

DISCUSSION

This paper describes a new nuclear acidic protein antigen, provisionally called MA, which appears distinct by immunodiffusion, nuclear immunofluorescent pattern, and physicochemical properties from the Sm (1), n-RNP (2), PM-1 (3), SS-A (4), and SS-B (5) nuclear antigens previously described.

The MA antibody appears to identify SLE patients with very severe manifestations of the disease. When compared with simultaneously studied groups of SLE patients having Sm antibodies or DNA antibodies, the SLE patients with MA antibodies had a particularly severe form of the disease, and may represent a subset of SLE patients.

The alternating presence in the circulation of the antigen and antibody suggests a biologic role for the MA system, perhaps similar to that of DNA anti-DNA immune complexes in SLE nephritis (6). Although the MA antigen has not yet been demonstrated by direct immunofluorescence or elution studies in kidney or other organs, these studies are pending and should be key to defining a biologic role for the MA system.

ACKNOWLEDGMENTS

The authors wish to thank Ms. Dianne Porter for her secretarial assistance and Mr. Lawrence G. Kingsland, III for statistical analysis.

This work was supported in part with grants from the Arthritis Foundation, National Institutes of Health (AM 20305A), and Clinical Research Center (RR-00-287-12).

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