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Antibodies to histones of the IgG and IgM class in systemic lupus erythematosus

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SUMMARY

Antibodies to histones were measured by an ELISA. Histone antibodies of the IgM class could be detected in other diseases than SLE, as well as in healthy persons. However, IgG histone antibodies proved to be specific for SLE and could be found in 21% of the SLE patients. These antibodies were highly associated with dsDNA antibodies of the IgG class. We could not find any correlation between IgG histone antibodies and clinical symptoms of SLE. IgG histone antibodies mostly reacted equally with all tested histone fractions.

Keywords histones antibodies systemic lupus erythematosus ELISA

INTRODUCTION

Antibodies to histones are heterogeneous. Some react with the isolated histones, others with the DNA–histone complex as it is present in the core mononucleosomes. Those reacting with DNA complexed histones show unusual cross-reactivities. They exhibit rheumatoid factor activity (Hannestad & Johanessen, 1976; Agnello *et al.*, 1980; Hannestad & Stollar, 1978), they react with plasma membranes (Rekvig & Hannestad, 1979, 1980) and induce the formation of lupus erythematosus (LE) cells (Rekvig & Hannestad, 1981). Antibodies to histones and histone fractions—not complexed with DNA—had been demonstrated with various techniques such as complement fixation (Stollar, 1971), immunofluorescence on reconstituted tissue sections (Fritzler & Tan, 1978; Fishbein, Alarcon-Segovia & Vega, 1979), enzyme linked immunoassay (ELISA) (Aitkaci, Monier & Mamelle, 1981) and solid phase radioimmunoassay (Blankstein, Stollar & Levy, 1980; Rubin, Joslin & Tan, 1982). The results differ widely with regard to systemic lupus erythematosus (SLE) specificity and reactivity with the different histone fractions, as will be discussed later.

In the present communication we describe an ELISA for histone antibodies of the IgG and IgM class. The antibodies were tested for their reactivity with the total histones as well as the different histone fractions. When sera of patients with SLE, rheumatoid arthritis, scleroderma or other diseases were tested, only the IgG histone antibodies proved to be SLE specific, while those of the IgM class could be detected in non-SLE sera as well as in sera of healthy persons.

MATERIALS AND METHODS

Sera. Sera were obtained from patients with SLE (63), mixed connective tissue disease (MCTD) (4), overlap syndromes (3), rheumatoid arthritis (RA) (24), scleroderma (Scl) (8), various other

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diseases (28) and healthy persons (23). Of the SLE patients group 60 fullfilled the ARA criteria (Tan *et al.*, 1982), three presented only three criteria and were judged as 'probable SLE'. The sera were frozen at the day the blood was drawn and stored at −20°C.

Chemicals. Histones and histone fractions (H1, H2a-H4, H2b, H4) (Sigma), Lambda DNA (BRL-Lab), bovine serum albumin (BSA) (fraction V, Sigma), poly-L-lysine (mol. wt 60,000, Sigma), alklaline phosphatase conjugated anti-human IgG serum (Behring), alkaline phosphatase conjugated anti-human IgM serum (Behring), IgG coated latex particles (Rapitex-RF, Behring), *p*-nitrophenylphosphate and 1M diethanolamine-HCL buffer were obtained from Merck.

ELISA for histone antibodies. According to the results presented later, the following procedure was used as the routine assay: 50 μl of a 50 μg/ml solution of histones (or histone fractions) were introduced into each well of the flat bottom polystyrene microtitre plate (Dynatech) and incubated at 37°C for 2 h. Then the plates were washed five times with 0·9% NaCl using an automatic washer (Dynatech) and 50 μl of 0·1 M Tris-HCl, pH 7·3, 2% BSA (BSA-Tris) were added to the wells. After incubation for 2 h at 37°C for 2 h the buffer was flicked off and the plates were dried at room temperature. The histone coated plates could be stored for at least 2 weeks at 5°C in a dry atmosphere without loss of reactivity.

Detection of antibodies. The sera to be tested were diluted 1:100 in BSA-Tris. The histone coated plate was filled with 50 μl diluted serum per well in duplicates. The first two rows were used for a highly positive standard serum in serial dilutions. The plates were incubated for 2 h at 37°C and washed five times with 0·9% NaCl. Fifty microlitres of anti-human IgG or anti-human IgM conjugated with alkaline phosphatase were pipetted into each well. These antisera are pre-titred by the manufacturer and were used in the recommended dilution (in BSA-Tris). The antisera were incubated for 2 h at 37°C and washed five times with 0·9% NaCl. One hundred microlitres of a 10 mM *p*-nitrophenyl solution in 1 M diethanolamine-HCl buffer, 0·5 mM $MgCl_2$, pH 9·4 (both from Merck) were added per well. After 10–15 min at room temperature the reaction was stopped by adding 100 μl of 2N NaOH. The absorption was measured by using a microelisa reader (Dynatech) with the unreacted substrate solution as blank.

ELISA for dsDNA antibodies. The assay was described before (Krippner, Merle & Pirlet, 1983). This test measures IgG dsDNA antibodies reacting with lambda DNA.

Absorption of rheumatoid factor activity. Sera were diluted 1:25 with BSA-Tris and incubated with an equal volume of human IgG coated latex particles (Rapitex-RF, Behring) at 37°C. After incubation the sera were further diluted with BSA-Tris to a final dilution of 1:100.

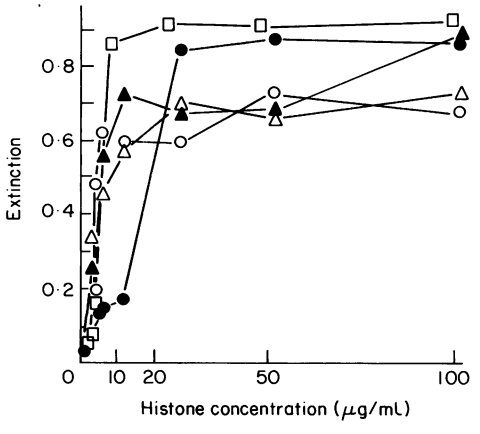

Fig. 1. Binding of histones and histone fractions to the polystyrene microtitre plate at different concentrations. The histones were measured in an enzyme immune reaction using a highly positive SLE serum. □ = histones; ▲ = H2a–H4; ● = H1; △ = H3; ○ = H2b.

RESULTS

Binding of histones

The binding of the histones and the histone fractions to the polystyrene plate was assayed by adsorbing the histones in various concentrations. The bound histones were detected in the ELISA with a serum, which is highly positive for histone antibodies. As shown in Fig. 1 the binding sites on the plate were saturated at a concentration of 25 μg/ml; some fractions were already saturated at 12·5 μg/ml.

Standardization

The standard curve of a highly positive SLE serum for histone antibodies of the IgG class is shown in Fig. 2. The day-to-day variation for the slope and range of the standard curves was less than 10%.

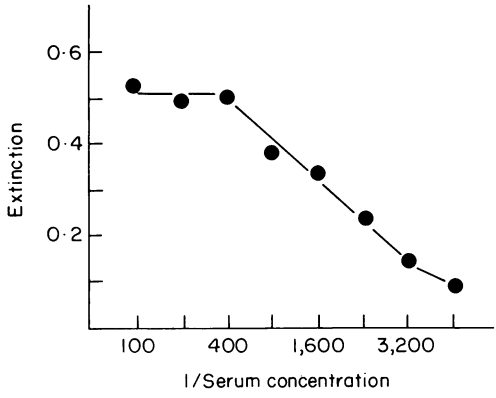

Fig. 2. Standard curve for IgG histone antibodies for a highly positive SLE serum.

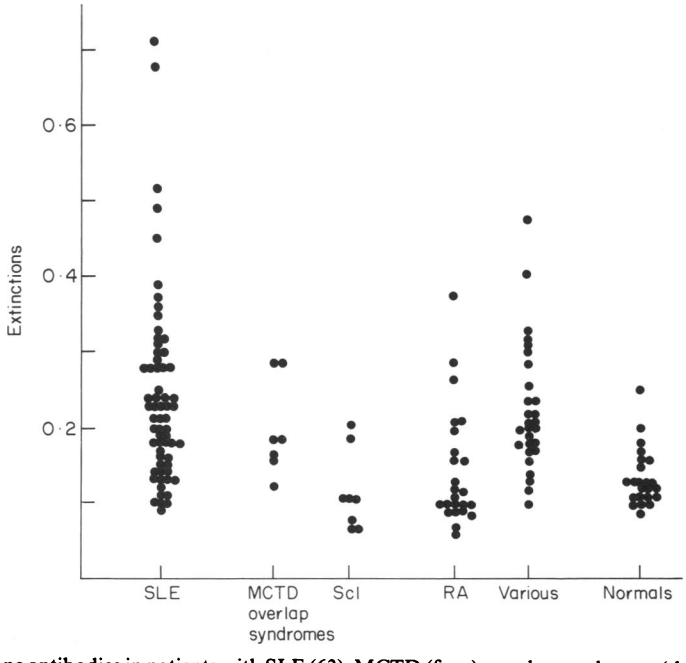

Fig. 3. IgM histone antibodies in patients with SLE (63), MCTD (four), overlap syndromes (three), scleroderma (eight), RA (24), various diseases (28) and healthy persons (22).

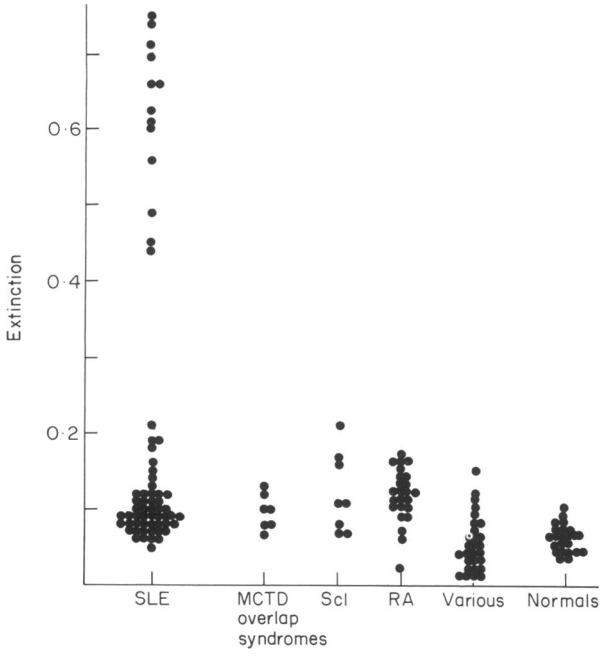

Fig. 4. IgG histone antibodies in patients with SLE (63), MCTD (four), Overlap syndromes (three), scleroderma (eight), RA (24), various diseases (28) and healthy persons (22).

It was not attempted to evaluate the absolute amount of histone antibodies. The results are given as the extinction measured.

Patient studies

IgM histone antibodies were not SLE specific (Fig. 3). High values were found for other diseases than SLE as well as in normal persons.

IgG histone antibodies were highly specific for SLE (Fig. 4). All positive sera showed high values (more than ext. = 0·44) while negative sera remained below 0·22. The three sera of patients with 'probable' SLE were among the 13 positive sera.

The patients who were positive for IgG histone antibodies were tested for their reactivity with the isolated histone fractions. The results are demonstrated in Table 1.

Ten of the 13 sera positive for IgG histone antibodies reacted with all histones, only three patients showed a preferential binding to certain histone fractions.

Correlation of histone antibodies with dsDNA antibodies

The SLE sera were tested for IgG dsDNA antibodies by use of an ELISA as described before. IgG dsDNA antibody concentrations over 8 μg/ml were considered as specifically elevated for SLE ('positive'). The appearance of IgG histone antibodies showed high correlation with IgG dsDNA antibodies: 11 of the 13 sera positive for IgG histone antibodies were positive for IgG dsDNA antibodies too.

Correlation of IgG histone antibodies with clinical symptoms

IgG histone antibodies were examined for their association with following clinical symptoms of SLE: arthritis, dermatitis, Raynaud's syndrome, cutaneous vasculitis, nephritis, haematological symptoms, sicca syndrome. The symptoms were divided into two groups—those which had appeared at any time during the course of SLE and those which were present at the time when the blood was drawn. IgG histone antibodies did not correlate with any of the SLE symptoms.

Table 1. Sera positive for IgG histone antibodies were tested against the isolated histone fractions

	Total histones	H1	H2a–H4	H2b	H3
Definite SLE					
0·68	1	0·49	0·60	0·63	0·66
2	0·56	0·69	0·70	0·63	0·74
3	0·61	0·57	0·66	0·63	0·56
4	0·66	0·65	0·77	0·75	0·74
5	0·66	0·67	0·85	0·72	0·69
6	0·69	0·65	0·80	0·88	0·74
7	0·60	0·59	0·65	0·70	0·64
8	0·75	0·60	0·90	0·81	0·88
9	0·74	0·64	0·83	0·72	0·79
10	0·45	0·59	—*	0·58	—
Probable SLE					
11	0·44	0·53	0·48	0·40	—
12	—	—	0·51	—	—
13	0·72	0·70	0·88	0·74	0·86

* Extinction below 0·25.

SLE activity and IgG histone antibodies

From 58 SLE patients, sufficient clinical information was available to estimate the disease activity by the disease activity index as described before (Krippner *et al.*, 1983). Symptoms of SLE activity were rated by a designated amount of points. The sum of the points for each symptom present at the time the serum was drawn was defined as the activity index.

The patients were divided into three groups according to the activity index at the time the blood was drawn (Table 2). Group A consisted of patients whose activity index was 10 or less. They showed no symptoms of SLE at all or only constant minor symptoms like chronic leucopenia. These patients were designated 'inactive'. Group B consisted of patients with 'minor activity'. They showed minor exacerbations of the disease such as arthritis, dermatitis, or migraine like symptoms. In group C, all patients were registered who experienced a major exacerbation like nephritis, necrotizing vasculitis and epilepsy during the time of the study. This group was defined to have 'major activity'.

The IgG histone antibodies did not show a significant association with disease activity. On the other hand, in some patients, the histone antibody concentration followed the SLE activity during the course of the disease as is demonstrated in Fig. 5. Under steroid treatment the antibody concentration fell rapidly. During exacerbation, the antibodies of the IgM class (which were already rather high from the start) showed fluctuations parallel to those of the IgG class. With regard to the IgG antibodies of the different histone fractions, we found (in this patient) that only the antibodies to the H1, H3 and H2B fractions were positive, while those to the H2a–H4 fraction remained low.

Table 2. Patients groups of SLE-activity

Group	Number	Activity	IgG histone antibody positive	IgG dsDNA antibody positive
A	35	low	9 (26%)	18 (51%)
B	17	medium	5 (29%)	11 (64%)
C	6	high	1 (17%)	5 (83%)

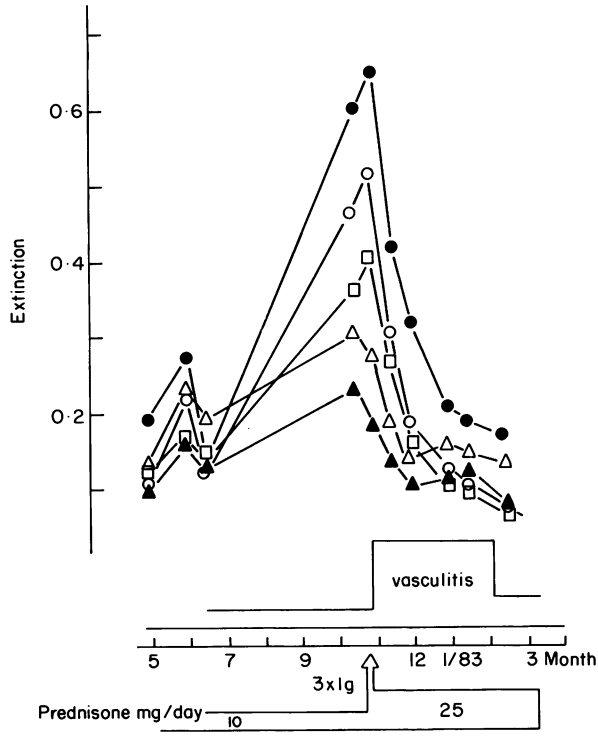

Fig. 5. IgG antibodies to total histones and histone fractions during the course of a patient with SLE. ● = H1; △ = H3; ▲ = H2a–H4; ○ = H2B; □ = histones.

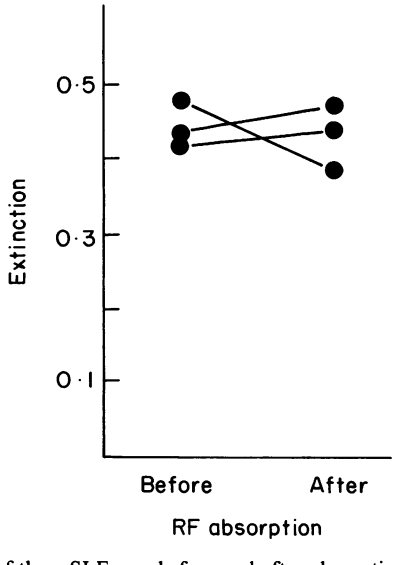

Fig. 6. IgG histone antibodies of three SLE sera before and after absorption with IgG coated latex particles

Rheumatoid factor activity

Antibodies to nucleosomes are known to have rheumatoid factor activity. Three sera, highly positive for IgG histone antibodies, were absorbed with IgG coated latex particles. As shown in Fig. 6 this treatment did not influence the reactivity of the sera in the IgG histone assay. This indicates that the IgG histone antibodies are not identical to those reacting with the histones as they are arranged in the nucleosomes.

DISCUSSION

Antibodies to histones can be separated into two groups, those reacting with free, isolated histones and those reacting with the histones, which are bound with DNA in the nucleosomes. These anti-nucleosome antibodies show various cross-reactivities. They react similar to rheumatoid factors with IgG (Hannestad & Johanessen, 1976; Agnello *et al.*, 1980; Hannestad & Stollar, 1978), as well as with plasma membranes (Rekvig & Hannestad, 1979, 1980) and are responsible for the appearance of LE cells (Rekvig & Hannestad, 1981). These cross-reactivities lead us to expect that these antibodies should not have any SLE specificity. As expected, they could be demonstrated in 44% of the rheumatoid factor positive sera (Agnello *et al.*, 1980).

The antibodies to the isolated histones however show a rather high specificity for SLE, although the results are highly divergent concerning the reactivity with certain histone fractions and the frequency of positive results in SLE. We used histones from calf thymus prepared by salt extraction (Sigma), and found 21% of the SLE patients positive for IgG histone antibodies. One group (Aitkaci *et al.*, 1981; Gioud, Aitkaci & Monier, 1982) used the same histone preparations in an ELISA but measured histone antibodies without separating the immunoglobulin classes. They found 52% of the SLE sera positive.

The differences found in the reactivity of histone antibodies with histone fractions are impressive. We did not find any preferential reactivity with any histone fraction, 10 of the 13 positive sera reacted with all fractions. Aitkaci *et al.* (1981), using the same histone preparations, found that SLE sera reacted primarily with the fractions H1 and H2B. In a solid phase radioimmunoassay Rubin *et al.* (1982) found (using histione fractions isolated by themselves) SLE sera to react mostly with the H3–H4 complex and the H2A–H2B complex. These complexes are not present in the preparation from Sigma. Stollar (1971) found histone antibodies directed primarily against the H1 fraction using a complement fixing assay. The contradictions revealed by these studies may be due, at least for a certain degree, to the different methods of preparing histones. However, there might be other reasons as well, as the differences in the molecular structure of the histones. These proteins are partially highly conserved throughout the different species. On the other hand there are parts of the histone molecules which show inter- and intraspecies variations, and, furthermore, the histones are modified by post-synthetical chemical reactions such as methylation and phosphorylation (reviewed by McHee & Felsenfeld, 1980). These variations in histone structure might have been the reason for the diverging results concerning histone antibodies.

The SLE specificity of IgG histone antibodies and the non-SLE specificity of IgM histone antibodies described by Rubin *et al.* (1982) is confirmed by our study. This corresponds to the finding that autoantibodies of the IgM class are very common in healthy persons (Daar & Fabre, 1981).

The antibodies found in this study are not identical to those reacting with nucleosomes, since the absorption of IgG histone antibodies with IgG coated latex particles did not alter the reactivity of the sera as would have been expected if the antibodies were directed to the nucleosomes. Antibodies to histones had been described in patients with drug-induced SLE by means of a solid phase radioimmunoassay (Portanova *et al.*, 1982). We could not verify this, since none of our SLE patients had drug-induced SLE. Some studies had been undertaken with histone reconstituted tissue sections (Fishbein *et al.*, 1979; Fritzler & Tan, 1978). With this technique it seems possible that a reassociation of DNA and histones to nucleosomes might occur. For this reason, the differentiation between antibodies to histones and those to nucleosomes would not have been possible, and the results of these studies were not comparable with those, using isolated histones.

The diagnostic value of the IgG histone antibodies, as measured with the assay described here, seems to be low, since in most sera they correlate with the IgG dsDNA antibodies. The IgG dsDNA antibodies show much higher sensitivity than the IgG histone antibodies. Aside from this, we saw no correlation between IgG histone antibodies and clinical symptoms.

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